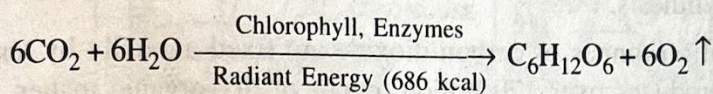
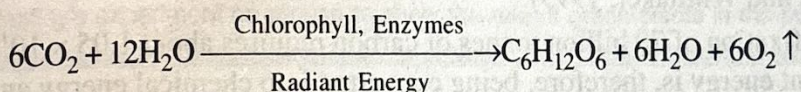


Photosynthesis (Gk. *phos* or *photos*— light, *synthesis*— putting together) is an enzyme regulated anabolic process of manufacture of organic compounds inside the chlorophyll containing cells from carbon dioxide and water with the help of sunlight as a source of energy. A simple equation of photosynthesis is as follows :



However, the function of water is to provide hydrogen for the synthesis of organic compounds. All the liberated oxygen comes from it. Therefore, the equation is modified as



The organisms which perform photosynthesis are called photoautotrophs. They include green plants, red algae, brown algae, yellow green algae, several types of protists, cyanobacteria and some bacteria. Phototrophic bacteria employ hydrogen donor other than water. Therefore, photosynthesis is also defined as an enzyme mediated anabolic process of manufacture of organic compounds inside the chlorophyll containing cells from carbon dioxide and hydrogen donor with the help of radiant energy.

Importance

It is the **most important natural process** which sustains life on earth.

1. The process of photosynthesis is unique to phototrophs which are mainly autotrophic plants. It synthesises organic food from inorganic raw materials.
2. All animals and heterotrophic plants depend upon the phototrophs for their organic food (all flesh is green). Because of it the phototrophs are called **producers**, while all other organisms are known as **consumers**.
3. Photosynthesis converts radiant or solar energy into chemical energy. The same gets stored in the organic food as bonds between different atoms. Photosynthetic products provide energy to all organisms to carry out their life activities (all life is bottled sunshine).
4. Coal, petroleum and natural gas are **fossil fuels** which have been produced by the application of heat and compression on the past plant and animal parts (all formed by photosynthesis) in the deeper layers of the earth. They are important source of energy to run many of our machines.
5. All useful plant products are derived from the process of photosynthesis, e.g., timber, rubber, resins, drugs, oils, fibres, etc.
6. It is the only known method by which oxygen is added to the atmosphere to compensate for oxygen being used in the respiration of organisms and burning of organic fuels. Oxygen is important in (a) efficient utilisation and complete breakdown of respiratory substrate and (b) formation of ozone in stratosphere that filters out and stops harmful UV radiations in reaching earth.

7. Photosynthesis decreases the concentration of carbon dioxide which is being added to the atmosphere by the respiration of organisms and burning of organic fuels. Higher concentrations of carbon dioxide are poisonous to living beings.

8. Productivity of agricultural crops directly depends upon the rate of photosynthesis. Therefore, scientists are busy in genetically manipulating the process.

Magnitude of Photosynthesis

The total carbon dioxide available to plants for photosynthesis is about 11.2×10^{14} tonnes. Out of this only 2.2×10^{13} (2200 billion) tonnes are present in the atmosphere at the rate of 0.03% replenishment. The amount is sufficient to support photosynthesis for a few hundred years even if there is no replenishment. Oceans contain 11×10^{14} or 110,000 billion tonnes of carbon dioxide. There is still a large quantity of carbon (2.8×10^{18} tonnes) present in the lithosphere. Of course, it is not employed in photosynthesis.

About 70–80 billion tonnes of carbon dioxide are fixed annually by terrestrial and aquatic photoautotrophs. It produces over 170 billion tonnes of dry organic matter. According to older estimates out of this only 10% (17 billion tonnes) of dry matter is produced by land plants while 90% of it is formed in oceans. The present estimates put the productivity of land plants to be 68% of the total (Lieth and Whittaker, 1975).

The annual fixation of 70 billion tonnes of carbon requires about 1.05×10^{18} kcal of energy. So much of radiant energy is, therefore, being converted into chemical energy annually. The total solar energy falling on earth is 5×10^{20} kcal/yr. The plants are thus able to utilize only 0.2% of the solar energy received by the surface of earth.

History

1500 B.C. **Ancient Indians.** Plants are referred by the name 'Padapa', which means the ones who drink through their feet.

320 B.C. **Aristotle and Theophrastus.** Like ancient Indians, the two great philosophers thought that plants obtain all their nourishment directly from soil.

1648 **Van Helmont.** He found that in five years as a willow plant increased its weight to 33 times, the soil lost only 56.7 gm of matter. Van Helmont, therefore, thought that vegetation is only water.

1727 **Stephen Hales.** Recognised the importance of sunlight, air and green leaves for nourishment of plants.

1770 **Joseph Priestley** (1733–1804; Fig. 13.1). Priestley (1770) found that a burning candle would soon get extinguished in closed space of bell jar. Similarly, a mouse kept in closed space would soon get suffocated and die. However, if a mint plant accompanied them in bell jar, neither the candle would be extinguished nor the mouse died. The plant also grows. Priestley, therefore, hypothesised that foul air or **phlogiston** produced during burning of candles or animal (mice) respiration could be converted into pure air or **dephlogiston** by plants (mint). In 1774, Priestley discovered oxygen.

1779 **Jan Ingenhousz** (1730–1799). In his experiment with an aquatic plant, he showed that in bright sunlight, small bubbles were formed around the green parts while in the dark, such bubbles did not form. He found these bubbles to be of oxygen. He thus confirmed that purification of air or formation of dephlogiston is carried out by green plants only in the presence of sunlight.

1782 **Senebier.** The rate of purification of air by green plants depends upon the concentration of foul air.

1783 **Lavoisier.** Phlogiston is carbon dioxide. Dephlogiston is oxygen.

1804 **De Saussure.** Water is essential requirement in photosynthesis. In photo-synthesis, plants pick up CO_2 and release O_2 .

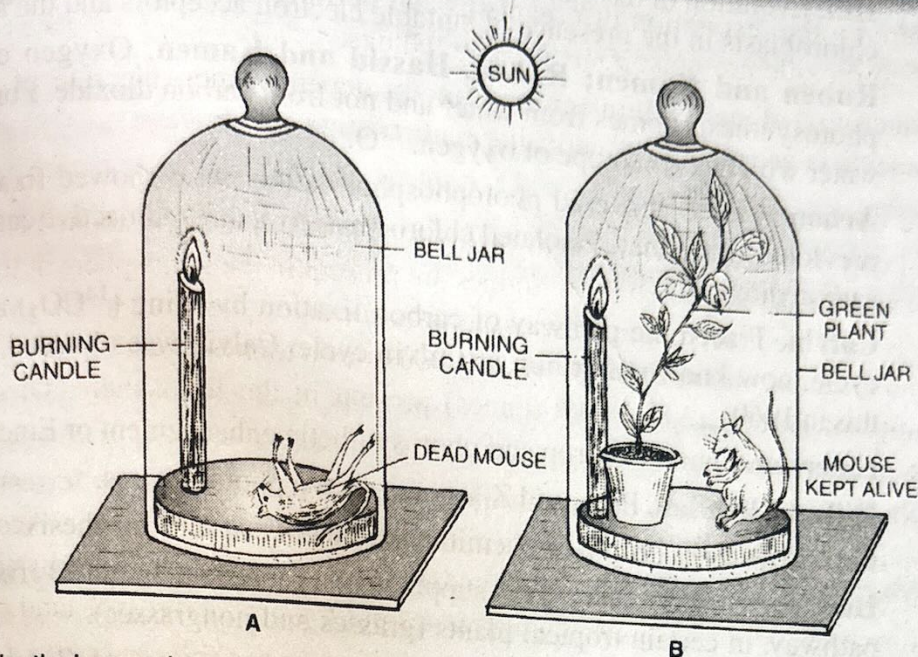
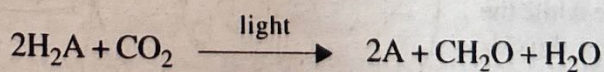


Fig. 13.1. Priestley's experiment on mouse to show the role of green plants in the purification of air.

- 1818 **Pelletier and Caventou.** Discovered and named chlorophyll.
- 1837 **Dutrochet.** Established connection between photosynthesis and chlorophyll.
- 1845 **Liebig.** Organic matter synthesised during photosynthesis is derived from carbon dioxide and water.
- 1845 **Von Mayer.** Green plants convert solar energy into chemical energy during the process of photosynthesis.
- 1854 **Sachs.** Green part in plant produces glucose which is stored as starch. Starch is the first visible product of photosynthesis.
- 1888 **Engelmann** (1843–1909). He split light into its components by the prism and then illuminated *Cladophora* (a green alga) placed in a suspension of aerobic bacteria. He found that bacteria accumulated in the region of blue and red light of the split spectrum. He thus discovered the effect of different wave lengths of light on photosynthesis and plotted the first **action spectrum** of photosynthesis.
- 1905 **Blackmann.** Propounded the 'law' or principle of limiting factors. He also proposed the occurrence of a dark phase in photosynthesis.
- 1920 **Warburg.** First employed *Chlorella*, a unicellular nonmotile green alga, for study of photosynthesis. He studied the effect of high O_2 concentration, poisons like cyanide and light flashes on photosynthesis.
- 1931 **van Neil** (1897–1985). On his studies with purple and green sulphur bacteria, demonstrated that photosynthesis is a light dependent reaction in which hydrogen from an oxidisable compound reduces CO_2 to form sugar.



In green sulphur bacteria, when H_2S , instead of H_2O was used as hydrogen donor, no O_2 was evolved. He inferred that O_2 evolved by green plants comes from H_2O but not from CO_2 as thought earlier.

- 1932 **Emerson and Arnold.** Performed flashing light experiments at different temperatures and found out the existence of light and dark phases of photosynthesis.

- 1937 **Hill.** Evolution of oxygen occurs in light reaction performed by isolated illuminated chloroplasts in the presence of suitable electron acceptors and the absence of CO_2 .
- 1941 **Ruben and Kamen; Ruben, Hassid and Kamen.** Oxygen evolved during photosynthesis comes from water and not from carbon dioxide. For this, they used water with heavy isotope of oxygen, ^{18}O .
- 1954 **Arnon et al.** Discovered photophosphorylation. Also showed fixation of CO_2 by previously illuminated isolated chloroplasts by using radioactive carbon ^{14}C in their carbon dioxide.
- 1954-55 **Calvin.** Traced the pathway of carbon fixation by using ($^{14}\text{CO}_2$) and gave the C_3 cycle, now known after him as Calvin cycle. Calvin was awarded Nobel Prize for this in 1960.
- 1957 **Emerson.** Found red drop and photosynthetic enhancement or Emerson effect.
- 1960 **Hill and Bendall.** Proposed Z-scheme of two photosystems.
- 1961 **Peter Mitchell.** Proposed chemiosmotic theory of ATP synthesis.
- 1965 **Hatch and Slack.** Discovered supplementary mechanism of CO_2 fixation, called C_4 pathway, in certain tropical plants (grasses and nongrasses).
- 1985 **Huber et al.** Crystallised and analysed the photocentre of *Rhodobacter* through X-ray diffraction technique. They were awarded Nobel Prize in 1988.

Raw Materials

1. **Carbon Dioxide.** In land plants, carbon dioxide is obtained from the atmosphere through the stomata. Small quantities of carbonates are also absorbed from the soil (Kursanov *et al.*, 1951) through the roots. Hydrophytes get their carbon dioxide supply from the aquatic environment as bicarbonates. Bicarbonates are absorbed by the hydrophytes through their general surface.

Experiment 1. Carbon Dioxide is necessary for Photosynthesis— Moll's Half Leaf Experiment.

Apparatus. A wide mouthed bottle, Potassium hydroxide solution, cotton, split cork, destarched potted plant, vaseline, apparatus for starch test (*see above*).

Procedure. Destarch a potted plant by keeping it in dark for 2-3 days. Insert apical half of one leaf in a wide mouthed bottle, containing KOH soaked cotton, by means of split cork. Place the apparatus in sunlight. Provide proper support to the bottle so that the leaf is not pulled. Remove the leaf under study and test for starch as given above.

Observations. Half of the leaf has turned bluish black. This part was outside the bottle. The apical half which was inside the bottle shows negative starch test.

Inference. Positive starch test indicates the occurrence of photosynthesis while negative starch test shows absence of photosynthesis. Both the halves of the leaf are receiving light and water. The apical half of the leaf is deprived of carbon dioxide while the outer half is receiving carbon dioxide from air. Inside the bottle all the carbon dioxide gets dissolved in potassium hydroxide. As the inner half kept in bottle is not performing photosynthesis, it proves that carbon dioxide is necessary for photosynthesis.

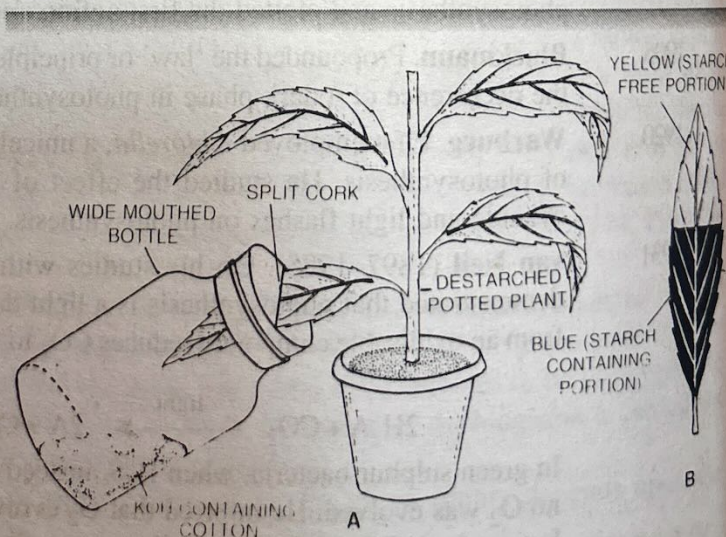
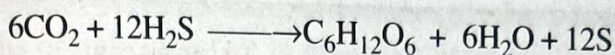


Fig. 13.2. Moll's Half Leaf Experiment to show that Carbon dioxide is necessary for Photosynthesis.

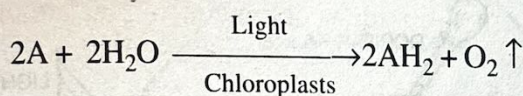
Precautions. (i) Leaf should not touch potassium hydroxide soaked cotton. (ii) Potassium hydroxide bottle should be provided with proper support. (iii) Make the connections air tight. (iv) Handle the leaf carefully during starch test. (v) Protect the spirit from catching fire through spilling or over heating.

2. **Water.** (i) van Niel (1931), while working on a type of photosynthetic bacteria, found that they required hydrogen sulphide for their carbon fixation. There was no evolution of oxygen. Sulphur globule accumulated as a waste product. Obviously in these photosynthetic bacteria, carbon dioxide did not split up as there was no evolution of oxygen. Hydrogen sulphide was broken down to provide hydrogen for reduction of carbon dioxide. As a result sulphur accumulates.



From the above observation and conclusions, Van Niel proposed that oxygenic photosynthesis of all organisms is actually an oxidation reduction reaction involving action of hydrogen of water over carbon dioxide to form organic compounds. He also propounded that oxygen is evolved from water.

(ii) Robin Hill (1937) illuminated the isolated chloroplasts of *Stellaria media* in the presence of leaf extract or hydrogen acceptors (e.g., ferricyanides, chromates, benzoquinones, dichlorophenol indophenol, etc.) in the absence of carbon dioxide. The chloroplasts evolved oxygen (Fig. 13.3).



where A represents hydrogen acceptor.

These hydrogen acceptors are also called **Hill oxidants** while the reaction involving the production of oxygen by the illuminated chloroplasts in the absence of CO_2 fixation is called **Hill reaction**. Vishniac and Ochoa (1951) found that the natural hydrogen acceptor of Hill reaction is NADP^+ (nicotinamide adenine dinucleotide phosphate).

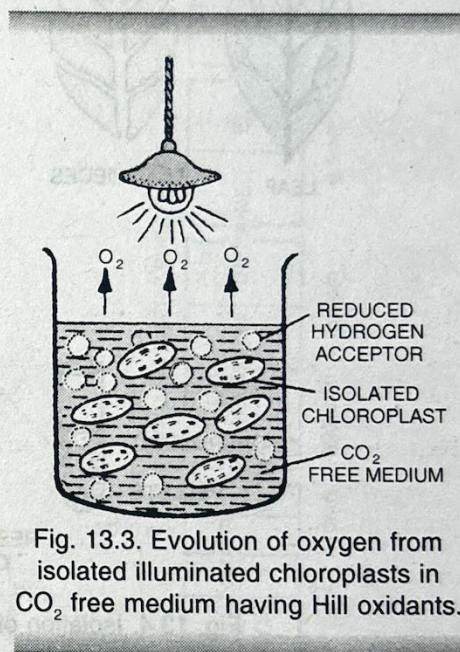
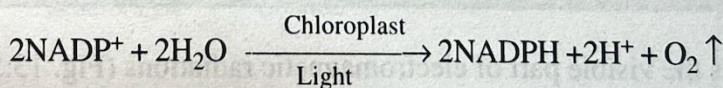


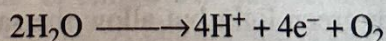
Fig. 13.3. Evolution of oxygen from isolated illuminated chloroplasts in CO_2 free medium having Hill oxidants.



Experiment 2. Demonstration of Hill Reaction (Fig. 13.4)

Apparatus. Well washed fresh non-mucilaginous leaves of Spinach or some other plant, 0.25M sucrose solution, homogeniser with cooling apparatus, centrifuge, muslin cloth, funnel, test tubes, dichlorophenol indophenol (DCPIP) solution.

Working. Veinless parts of a well washed fresh non-mucilaginous leaf are removed and homogenised in 0.25M sucrose solution. Homogeniser has a device for cooling and tearing the leaf parts to the level of suspension. It is filtered through muslin cloth. The filtrate is then centrifuged. The supernatant is discarded and the pellet or sediment is redispersed to obtain suspension of chloroplasts. Pure *Chlorella* culture can be used directly. A small quantity of oxidised or blue-coloured 2, 6-dichlorophenol indophenol is poured into the suspension. The suspension is now irradiated or exposed to light. The blue colour disappears. DCPIP becomes colourless only on being reduced. Simultaneously, oxygen bubbles can be observed to come out of the suspension. The gas bubbles can be collected and tested. This is possible only if chloroplasts have split up water (photolysis) into its components.



(iii) Ruben and Kamen (1941) and Ruben *et al* (1941) suspended *Chlorella* in water having nonradioactive heavy isotope of oxygen, ^{18}O , instead of natural oxygen, ^{16}O . The suspension was illuminated. Oxygen evolved was tested by means of mass spectrometer. It was found to be heavy isotope, ^{18}O . This is possible only if oxygen evolved during photosynthesis comes from splitting of water.

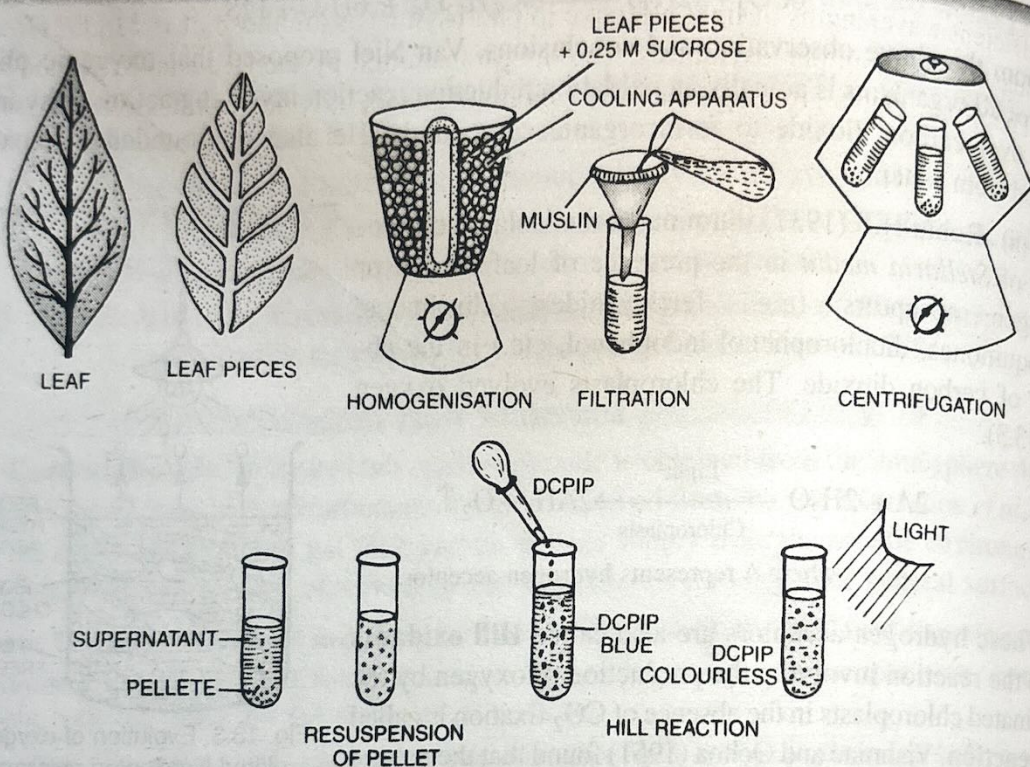
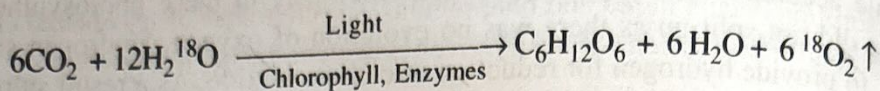


Fig. 13.4. Isolation of chloroplasts and demonstration of Hill reaction.

3. **Light.** Light is the visible part of electromagnetic radiations (Fig. 13.5). Electromagnetic radiations are a form of energy that consists of a stream of tiny particles which travel in waves. Depending upon the wavelength, electromagnetic spectrum consists of 8 types of radiations—cosmic rays, gamma rays, X-rays, ultra-violet radiations, light spectrum, infra-red rays, electric rays and radio waves. Visible light consists of radiations having a wavelength between 390–760 nm (or 3900–7600 Å). It can be resolved into light of different colours—**violet** (390–430 nm), **blue** or **indigo** of early workers (430–470 nm), **blue-green** or **blue** of early workers (470–500 nm), **green** (500–580 nm), **yellow** (580–600 nm), **orange** (600–650 nm), **orange-red** (650–660 nm) and **red** (660–760 nm). Red light above 700 nm is called **far-red**. Radiations shorter than those of violet are called **ultra-violet rays**. They have a wave length of 100–390 nm. Similarly, radiations longer than those of red are called **infra-red**. They have wave length of 760–100,00 nm. Sunlight or solar radiations reaching the earth have wavelength between 300 nm (in the ultraviolet range) to 2600 nm (in the infra-red range). Part of the spectrum used in photosynthesis has a wavelength between 400–700 nm. It is called **photosynthetically active radiation (PAR)**. Leaves appear green because chlorophylls do not absorb green light but allow the same to be reflected and transmitted through leaves.

Discrete particles believed to be present in light are called **photons**. Photons carry energy. The energy contained in a photon is termed as **quantum** ($h\nu$). The energy content of a quantum is related to its wavelength. The shorter the wavelength, the greater is the energy present in its quantum. Quantum energy is measured in units called **einsteins**.

80–90% of light falling on leaves is absorbed by them. The rest is reflected and transmitted. Most of the absorption occurs in red and blue regions. Green colour of leaves and chlorophyll is due to reflection and transmission of mostly green wavelengths of light.

Blue and red regions of the light spectrum are the most effective in photosynthesis. Blue wavelengths of light carry more energy while red wavelengths have lesser energy. Therefore, the most efficient wavelengths of light effective in photosynthesis are those of red light. Green light is the least effective in photosynthesis. The light transmitted by the tree canopy is rich in green light. Therefore, plants growing under the canopy of others have lower rates of photosynthesis.

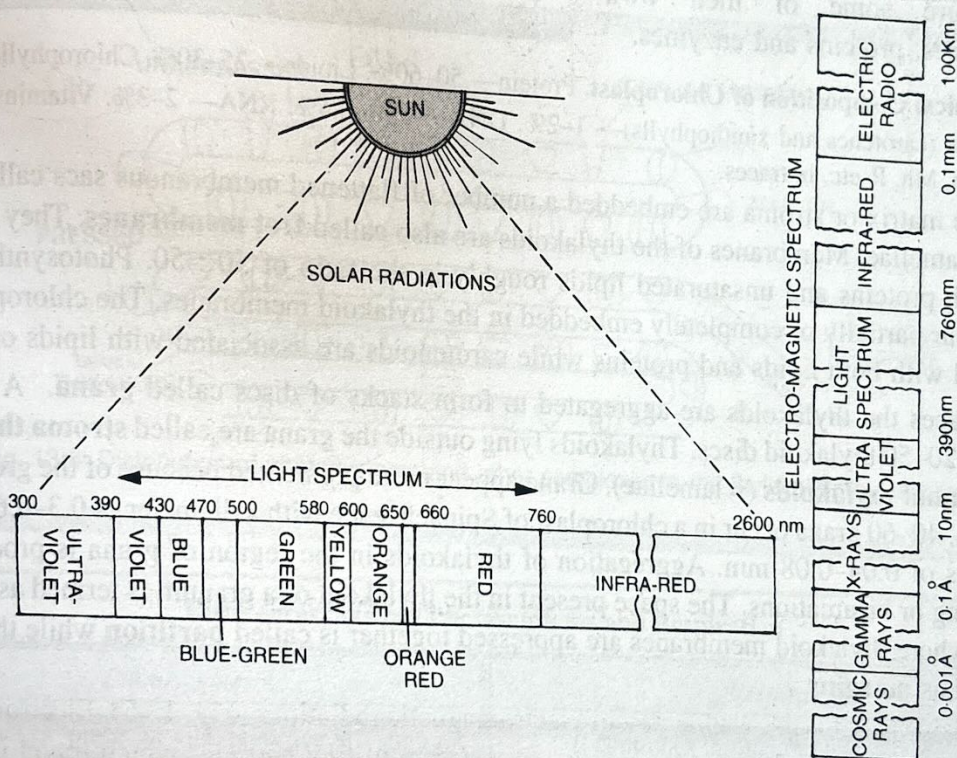


Fig. 13.5. Electromagnetic spectrum. Range of solar radiations and light spectrum.

4. Chloroplasts (Gk. *chloros*— green, *plastos*— moulded). Chloroplasts are green plastids which function as the **site of photosynthesis** in eukaryotic photoautotrophs. Leaves have the maximum number of chloroplasts with over half a million per square millimetre. Inside the leaves, the chloroplasts occur mostly in the mesophyll cells along their walls for easy diffusion of gases and receiving optimum quantity of incident light. The chloroplasts align themselves in vertical position along the lateral walls in high light intensity and along tangential walls in moderate light. The change in position helps in receiving optimum light by chloroplasts. A mesophyll cell may have as many as 300 chloroplasts. The latter are found in the peripheral cytoplasm.

In lower plants chloroplasts have diverse shapes and sizes. In higher plants the chloroplasts are disc-shaped with rounded, oval or ellipsoidal outline. Such chloroplasts measure 4–10 μ m in diameter with a thickness of 2–3 μ m. A chloroplast is covered by an envelope of two membranes, each of 9–10 nm thickness. They are separated by a translucent zone or periplastidial

space of 10–20 nm. Internally a chloroplast contains two structures, matrix or fluid stroma and membranous system called lamellar or thylakoids. The chloroplast **matrix** or **fluid stroma** is similar to cytoplasm in its constitution. It contains DNA, RNA, ribosomes, enzymes for CO_2 assimilation, proteins, starch grains and fat droplets or plastoglobuli. Chloroplast DNA or ctDNA is naked, circular or linear. It makes the chloroplasts semiauto-nomous. Ribosomes are of 70S type. With the help of ctDNA, RNA and ribosomes the chloroplasts manufacture some of their own polypeptides, proteins and enzymes.

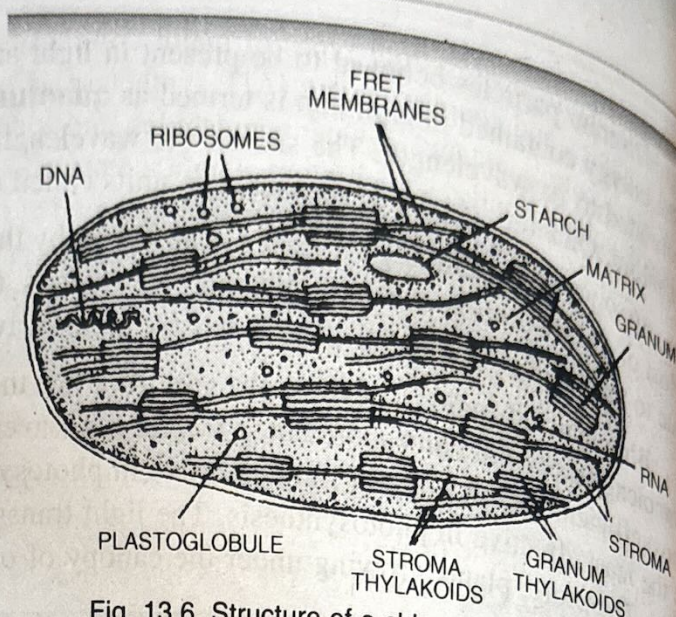


Fig. 13.6. Structure of a chloroplast in a section as seen under electron microscope.

Chemical Composition of Chloroplast. Protein— 50–60%. Lipids— 25–30%. Chlorophyll— 5–10%. Carotenoids (carotenes and xanthophylls)— 1–2%. DNA— upto 0.5%. RNA— 2–3%. Vitamins E and K, Mg, Fe, Co, Mn, P, etc. in traces.

In the matrix or stroma are embedded a number of flattened membranous sacs called **thylakoids** or lamellae. Membranes of the thylakoids are also called **fret membranes**. They are made up of both proteins and unsaturated lipids roughly in the ratio of 50 : 50. Photosynthetic pigments occur partially or completely embedded in the thylakoid membranes. The chlorophylls are associated with both lipids and proteins while carotenoids are associated with lipids only.

At places the thylakoids are aggregated to form stacks of discs called **grana**. A granum may have 20–50 thylakoid discs. Thylakoids lying outside the grana are called **stroma thylakoids** or **intergranal thylakoids** (= lamellae). Grana appear more pigmented because of the grouping of thylakoids. 40–60 grana occur in a chloroplast of Spinach, each with a diameter of 0.3–0.6 mm and a thickness of 0.04–0.08 mm. Aggregation of thylakoids in the region of grana is produced by their folding or bifurcations. The space present in the thylakoid of a granum is termed as **loculus**. The area where thylakoid membranes are appressed together is called **partition** while their sides are termed as **margins**.

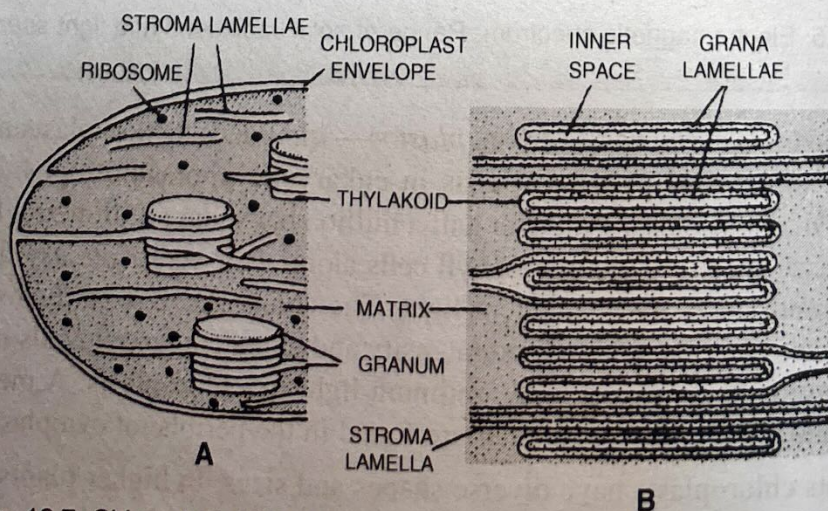


Fig. 13.7. Chloroplast thylakoids. A, part of the chloroplast showing stroma thylakoids and grana; B, arrangement of thylakoids in a granum.

Thylakoids possess four types of major complexes; photosystem I, photosystem II, Cyt b_6 -f complex and coupling factor (ATP synthetase). Components of photosystems consisting of reaction centres, antenna pigment molecules and electron transport molecules are associated (mostly noncovalently) with integral membrane proteins. The proteins project both into the stroma and locus with hydrophobic amino acids associated with membrane phospholipids. Photosystem II is thought to mostly occur in the appressed or partition regions of granal thylakoids while photosystem I lies in the nonappressed parts as well as stroma thylakoids. In addition, these parts also possess another complex called **coupling** factor. It is similar to F_0 - F_1 complex of mitochondria and is called CF_0 - CF_1 . Coupling factor takes part in photophosphorylation. Cyt b_6 -f complexes are evenly distributed in stroma and granal thylakoids.

A peripheral reticulum of tubules occurs below the chloroplast envelope in C_4 chloroplasts.

In photosynthetic prokaryotes (bacteria and cyanobacteria), chloroplasts or equivalent structures are absent. They, however, possess thylakoids. The latter lie freely in the cytoplasm. The pigments are different from those of eukaryotes.

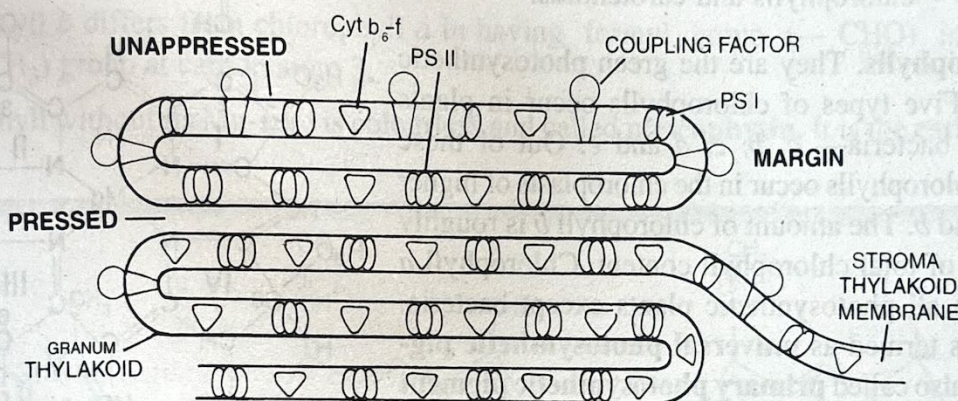


Fig. 13.8. Distribution of photosystems and other complexes in the thylakoid membranes.

Differences between Photosynthesis and Respiration

Photosynthesis	Respiration
1. It takes place in the presence of sunlight only.	1. It continues throughout day and night, in sun and darkness.
2. A plant can live without photosynthesis for a number of days.	2. No organism can live without respiration even for a few minutes.
3. Only green cells of the plant are capable of accomplishing photosynthesis.	3. All the cells of an organism perform respiration.
4. It absorbs sunlight and is an endothermic reaction.	4. It gives out energy and is, therefore, exergonic.
5. It utilises carbon dioxide and water.	5. It evolves carbon dioxide and water.
6. Photosynthesis is an anabolic process. It builds up complex compounds from simple inorganic compounds.	6. It is a catabolic process in which complex substances are broken down into simple substances.
7. It increases the weight of the organisms.	7. It decreases the weight of the organisms.
8. It changes radiant energy into chemical energy.	8. It releases the potential or chemical energy which is used variously in the body.
9. The raw materials of photosynthesis are carbon dioxide and water.	9. The raw materials of respiration are sugars and oxygen.

10. The end products are carbohydrates and oxygen.
11. Gaseous exchange involves release of oxygen and absorption of carbon dioxide.
12. It produces ATP by harvesting light energy.
13. Hydrogen of water is used in the synthesis of NADPH.
14. ATP and NADPH are employed in the synthesis of organic compounds.

10. The end products are carbon dioxide and water.
11. Gaseous exchange involves absorption of oxygen and evolution of carbon dioxide.
12. Respiration forms ATP from energy liberated during oxidation of food.
13. Hydrogen of food is used in the formation of NADH, NADPH and FADH_2 .
14. ATP and reduced coenzymes are made available to cells for various metabolic activities.

Photosynthetic Pigments

They are those pigments which occur on photosynthetic thylakoids of chloroplasts and take part in absorption of light energy for the purpose of photosynthesis. Photosynthetic pigments are of two main types—chlorophylls and carotenoids.

Chlorophylls. They are the green photosynthetic pigments. Five types of chlorophylls occur in plants other than bacteria—*a*, *b*, *c*, *d* and *e*. Out of these only two chlorophylls occur in the chloroplasts of higher plants, *a* and *b*. The amount of chlorophyll *b* is roughly one fourth of total chlorophyll content. Chlorophyll *a* is found in all photosynthetic plants except bacteria. Hence, it is termed as **universal photosynthetic pigment**. It is also called **primary photosynthetic pigment** because it performs primary reaction of photosynthesis which involves conversion of light into chemical or electrical energy. Other photosynthetic pigments are thence called **accessory pigments**. They absorb light energy of different wavelengths, broaden the spectrum of light absorption and hand over the energy to chlorophyll *a* through electron spin resonance. Some of the chlorophyll *a* molecules function as **reaction centres** (P_{700} , P_{680}). They bring about electrical charge separation and hence convert light energy into electrical energy.

Bacteria possess two types of related pigments—bacteriochlorophyll (further of several subtypes) and bacterioviridin (= chlorobium chlorophyll). Bacteriochlorophyll *a* has an empirical formula of $\text{C}_{55}\text{H}_{74}\text{O}_6\text{N}_4\text{Mg}$ and molecular weight of 911.

Chlorophyll *a* is bluish-green in the pure state. It has an empirical formula of $\text{C}_{55}\text{H}_{72}\text{O}_5\text{N}_4\text{Mg}$ and molecular weight of 893. Chlorophyll *b* is olive green in the pure state with an empirical formula of $\text{C}_{55}\text{H}_{70}\text{O}_6\text{N}_4\text{Mg}$ and molecular weight of 907. Both the chlorophylls are soluble in a number of organic

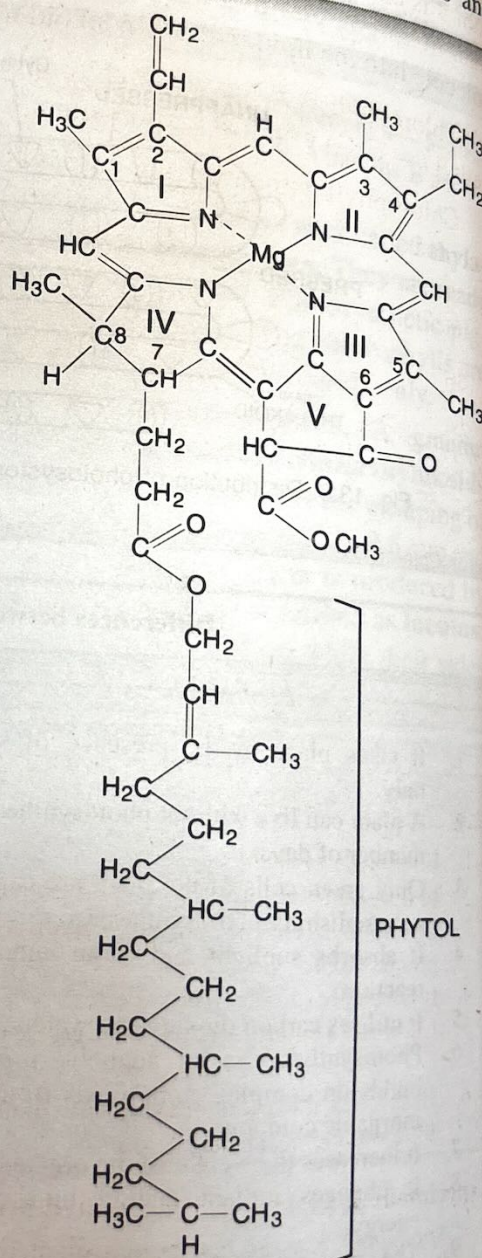


Fig. 13.9. Structure of chlorophyll *a*.

solvents but chlorophyll *a* is more soluble in petroleum ether while chlorophyll *b* is more soluble in 92% methyl alcohol.

Chlorophyll structure was first studied by Wilstatter, Stoll and Fischer in 1912. It has a tadpole like configuration with a head called **porphyrin** and a tail made up of long chain alcohol called **phytol** (Fig. 13.9). Porphyrin head is made up of four **pyrrole rings** which are linked by **methine bridges** ($-\text{CH}=\text{}$). The skeleton of each pyrrole ring is made up of 5 atoms—4 carbon and one nitrogen. The latter lies towards the centre. A nonionic magnesium atom is held in the centre of porphyrin head by nitrogen atoms of pyrrole rings (through two covalent and two coordinate bonds). The external carbon atoms of the pyrrole rings have been given specific numbers, 1-8. Carbon atoms 1, 3, 5 and 8 have methyl groups ($-\text{CH}_3$). Carbon atom 2 possesses a vinyl group ($-\text{CH}=\text{CH}_2$) while carbon atom 4 has an ethyl group ($-\text{CH}_2-\text{CH}_3$). Carbon atom 6 is attached to next methine group by a fifth isocyclic ring called cyclopentanone. Carbon atom 7 is connected to phytol tail through a propionic acid residue. **Phytol** is an insoluble long chain of carbon and hydrogen atoms with a formula of $\text{C}_{20}\text{H}_{39}\text{OH}$. It anchors the chlorophyll molecule into the lipid part of thylakoid membrane.

Chlorophyll *b* differs from chlorophyll *a* in having formyl group ($-\text{CHO}$) instead of a methyl ($-\text{CH}_3$) group at carbon atom 3.

Chlorophyll without its Mg-core is colourless and called **phaeophytin**. It is the early electron acceptor.

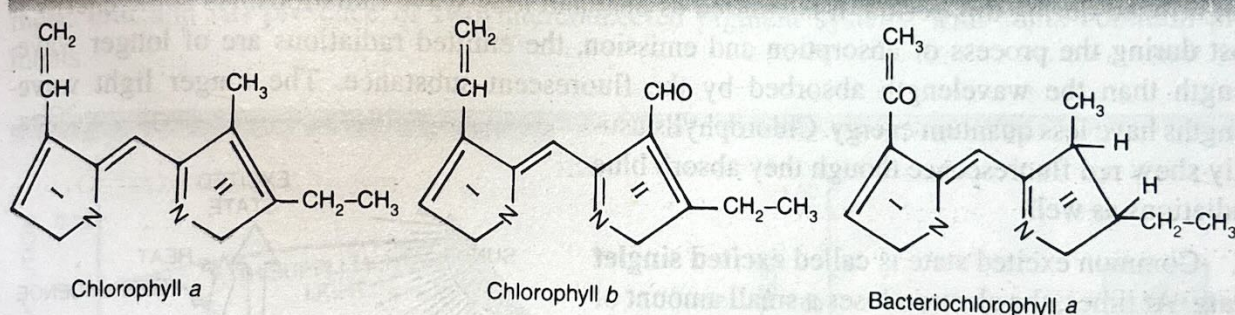


Fig. 13.10. Pyrroles I and II of chlorophyll *a*, chlorophyll *b* and bacteriochlorophyll *a* to show differences among them.

Differences between Chlorophyll *a* and Chlorophyll *b*

Chlorophyll <i>a</i>	Chlorophyll <i>b</i>
1. It is $\text{C}_{55}\text{H}_{72}\text{O}_5\text{N}_4\text{Mg}$. 2. In pure state, chlorophyll <i>a</i> is blue-green. 3. Chlorophyll <i>a</i> is primary photosynthetic pigment. 4. It is soluble in petroleum ether. 5. Carbon-3 contains methyl ($-\text{CH}_3$) group. 6. It absorbs more red wavelength than violet-blue wavelength of light.	1. It is $\text{C}_{55}\text{H}_{70}\text{O}_6\text{N}_4\text{Mg}$. 2. In pure state, chlorophyll <i>b</i> is olive-green. 3. It is accessory photosynthetic pigment. 4. It is soluble in 92% methyl alcohol. 5. Carbon-3 contains formyl or aldehyde ($-\text{CHO}$) group. 6. It absorbs more violet-blue wavelength than red wavelength of light.

Experiment 3. Light and Chlorophyll are necessary for Photosynthesis

Apparatus. Destarched potted plant of variegated Croton, black paper, apparatus for starch testing

Working. Take a destarched potted plant of Croton having variegated leaves (leaves with green and nongreen patches). Cover one leaf with black paper. Place the potted plant in sunlight. After two hours, pluck the unilluminated leaf and one illuminated leaf. Test both the leaves for starch.

Observation. The darkened or unilluminated leaf does not show any blue-black patch. The whole leaf appears yellowish after iodine test. The illuminated leaf possesses both blue-black and yellow patches. The bluish patches correspond to green areas while yellow patches are the ones which were previously nongreen.

Inference. Presence of bluish-black patches in illuminated leaf and their absence in darkened leaf clearly indicates that **light is necessary for photosynthesis**. In illuminated leaf only green or chlorophyll bearing parts appear bluish-black showing that **chlorophyll is necessary for photosynthesis**. The non-green patches do not perform photosynthesis.

Photoluminescence. It is the phenomenon of re-radiation of absorbed energy. Photoluminescence is of two types, fluorescence and phosphorescence. **Phosphorescence** is delayed emission of long wave radiations by irradiated substances which continues for some time after removal of irradiation source. Chlorophylls show mainly fluorescence. There is some delayed emission or phosphorescence but the same seems to be **chemiluminescence** or **bioluminescence**.

Fluorescence. It is the property of almost immediate emission of long wave radiations by a substance after having absorbed radiation energy. The substance which can emit back the absorbed radiations is called **fluorescent substance**. All photosynthetic pigments have the property of fluorescence. Chlorophylls show an outburst of fluorescence (called Kutusky effect) during the first few moments of illumination. However, most of the fluorescence emitted by photosynthetic organs is due to chlorophyll *a* because other pigments usually hand over their absorbed energy to it through resonance. On receiving light energy, chlorophyll gets changed to excited state. The excited state lasts for about 10^{-9} second. If the energy of the excited state is not utilized during this period, it is emitted as heat and long wave radiations. As some energy is lost during the process of absorption and emission, the emitted radiations are of longer wavelength than the wavelength absorbed by the fluorescent substance. The longer light wavelengths have less quantum energy. Chlorophylls usually show red fluorescence though they absorb blue radiations as well.

Common excited state is called **excited singlet state**. At times, the electron loses a small amount of energy and stays for some period in the less excited state called **triplet excited state**. Release of energy by the triplet excited state at the time of coming back to ground state is **phosphorescence** (delayed emission of long wave radiations from an irradiated and activated molecule). Sometimes the electron picks up more energy than the excited singlet state. It is called **second singlet state**. It remains in this state for a very brief period before coming to excited singlet state.

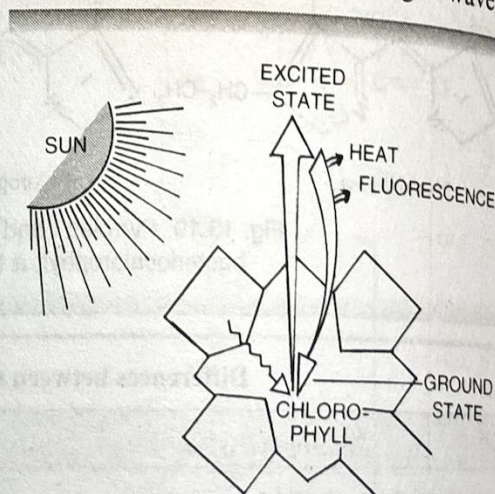


Fig. 13.11. Absorption of radiation, excitation and fluorescence by chlorophyll.

Experiment 4. Demonstration of Fluorescence by Chlorophyll (Fig. 13.12)

Apparatus. Soft fresh non-mucilaginous leaves of plant like Spinach, washed and dried fine sand, acetone, pestle and mortar, filtering apparatus, test tube, source of light.

Working. Take a few fresh non-mucilaginous leaves of plant like Spinach or *Chenopodium*. Wash them properly. Place the leaves in a mortar. Add a small quantity of fine washed and dried sand and 15–30 ml of acetone. Grind the leaves well with the help of pestle. Take out the coloured liquid and filter it. The filtrate is the solution of photosynthetic pigments, mainly chlorophylls. Pour it in a test tube. The test tube is placed between a source of white light and the observer. The colour of the solution is being observed through transmitted light. It is green. Now keep the test tube on one side of both the source of white light as well as

the observer. The colour of the solution will appear through reflected light or light that is being emitted by the solution. It is red.

Observation. Chlorophyll solution appears green in transmitted light because the green part of the light spectrum is not absorbed by the pigment. It appears reddish in reflected light because a part of the reflected light is due to fluorescence or light emitted by the pigment after absorbing the same. Therefore, chlorophyll exhibits fluorescence of red wavelength of light.

Emerson Effect. Emerson (1957) found a sharp reduction in the rate of photosynthesis when monochromatic beam of more than 680 nm was used alone. It is called **red drop** (Fig. 13.13). Emerson *et al* (1957) found that rate of photosynthesis can be enhanced if monochromatic beams of two different wavelengths (long and short) were applied simultaneously. It is in excess of sum total of photosynthesis carried out separately by two light beams. The phenomenon is called **Emerson effect** or **photosynthetic enhancement**. It is due to (i) presence of different types of harvesting molecules around a trap centre in a photosynthetic unit and (ii) presence of two interconnected pigment systems with some common pigments.

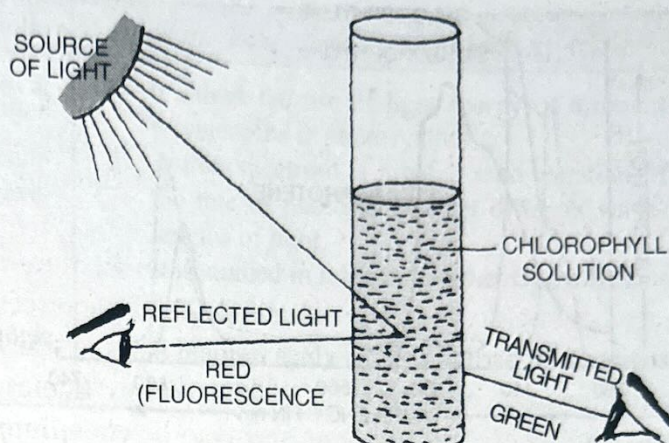


Fig. 13.12. Appearance of chlorophyll in transmitted and reflected light.

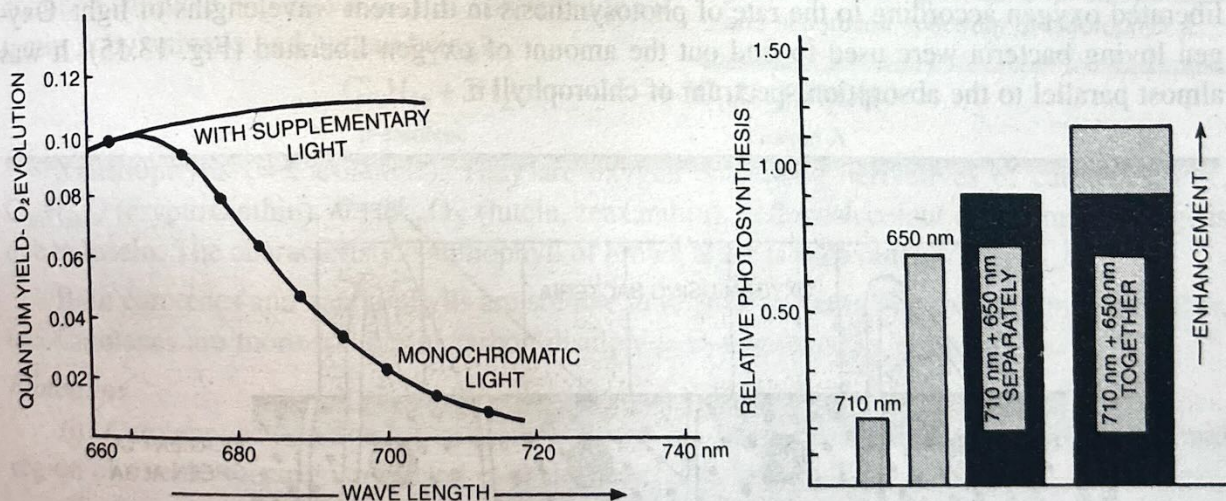


Fig. 13.13. Emerson Effect. A, graphic representation of red drop and photosynthetic enhancement with supplementary light. B, histograms showing relative photosynthesis at two different wavelengths employed separately and together.

Absorption Spectrum. The curve showing the amount of energy of different wavelengths of light absorbed by a substance is called graphic **absorption spectrum**. It is studied with the help of **spectrophotometer**. The absorption spectra of chlorophylls *a* and *b* (Fig. 13.14) show that they absorb maximum light in the blue-violet and red wavelengths. The pigments are often known after the wavelength which is absorbed to the maximum, e.g., Chl *a*₆₇₃, Chl *a*₆₈₃(P₆₈₀), Chl *a*₇₀₃(P₇₀₀).

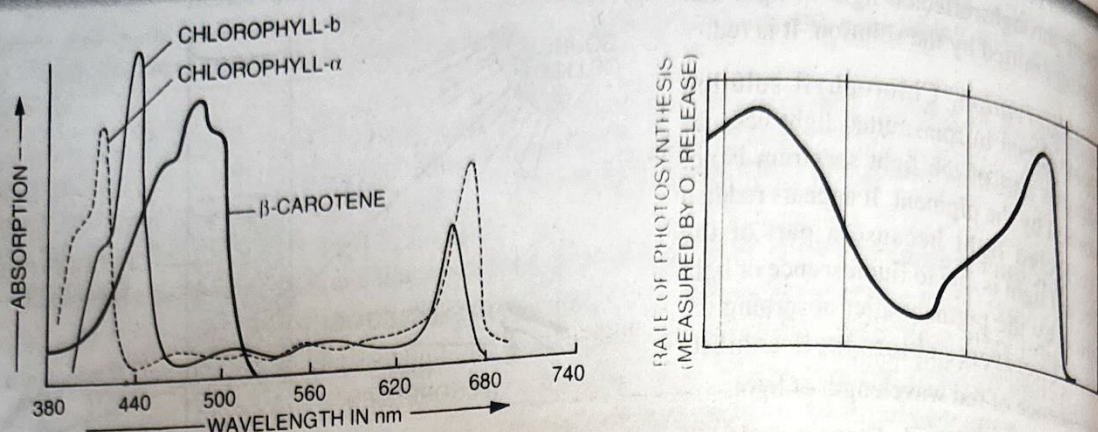


Fig. 13.14. Absorption and action spectra. A, absorption spectra of three photosynthetic pigments (chl a, chl b and β -carotene). B, action spectrum of photosynthesis (relative rates of photosynthesis at different wavelengths of light) is measured by oxygen release.

Action Spectrum. The graphic curve depicting the relative rates of photosynthesis at different wavelengths of light is called action spectrum (Fig. 13.14 B). It shows that maximum photosynthesis occurs in blue-violet and red parts of the light. Action spectrum of photosynthesis corresponds closely to absorption spectra of chlorophylls *a* and *b* showing that the latter are the main photosynthetic pigments. However, sufficient photosynthesis occurs in the mid part of the light spectrum where carotenoids (carotenes and xanthophylls) are active.

The first action spectrum was studied by Engelmann (1882) by using a green algae which liberated oxygen according to the rate of photosynthesis in different wavelengths of light. Oxygen loving bacteria were used to find out the amount of oxygen liberated (Fig. 13.15). It was almost parallel to the absorption spectrum of chlorophyll *a*.

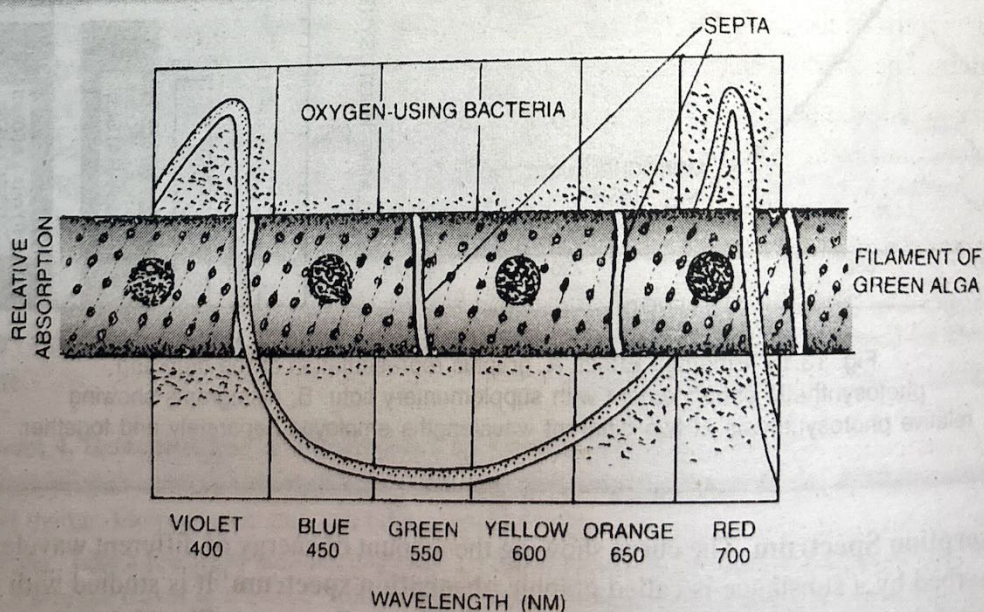


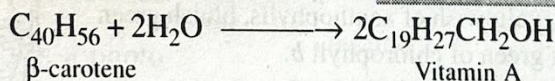
Fig. 13.15. Action spectrum of photosynthesis as determined by Engelmann (1882) with the help of oxygen loving bacteria.

Differences between Absorption Spectrum and Action Spectrum

Absorption Spectrum	Action Spectrum
1. It shows the amount of light absorbed at different wavelengths. 2. Absorption spectrum is graphic representation of amount of light of different wavelengths absorbed by a pigment. 3. It is studied directly. 4. Absorption spectrum requires a single exposure to full light.	1. It shows the use of light energy of different wavelengths in photosynthesis. 2. Action spectrum is graphic representation of the rate of photosynthesis at different wavelengths of light. 3. It is studied in relation to either O₂ evolved or CO₂ absorbed. 4. It requires study of light utilisation at various wavelengths.

Carotenoids. They are a group of yellow, brown to reddish pigments which are associated with the chlorophylls inside the chloroplasts but occur alone inside the chromoplasts. Along with chlorophyll *b* the carotenoids are also called **accessory pigments** because they hand over the energy absorbed by them to chlorophyll *a*. Carotenoids have conjugate double bonds ($-\text{C}=\text{C}-\text{C}=\text{C}-$). They are of two types, carotenes and xanthophylls.

Carotenes. They are hydrocarbons with a general formula of $\text{C}_{40}\text{H}_{56}$. Red colour of Tomato and Chillies is due to carotene called **lycopene**. The most common carotene is β -carotene. It is converted to vitamin A by animals and human beings.



Xanthophylls (= Carotenols). They are oxygen containing derivatives of carotenes, e.g., $\text{C}_{40}\text{H}_{56}\text{O}$ (cryptoxanthin), $\text{C}_{40}\text{H}_{56}\text{O}_2$ (lutein, zeaxanthin). Yellowish colour of autumnal foliage is due to **lutein**. The characteristic xanthophyll of brown algae is **fucoxanthin** ($\text{C}_{40}\text{H}_{56}\text{O}_6$).

Both carotenes and xanthophylls are soluble in organic solvents like chloroform, ethyl ether, etc. Carotenes are more soluble in carbon disulphide as compared to xanthophylls.

Functions

(i) Carotenoids function as accessory pigments. They absorb radiant energy in the mid region of visible spectrum and hand over the same to chlorophyll.

(ii) They protect the chloroplast constituents from nascent oxygen released during photolysis of water. Carotenoids pick up nascent oxygen by means of their double bonds and change the same into harmless molecular state.

(iii) Unquenched excited state of chlorophyll reacts with molecular oxygen to form a highly damaging excited state of oxygen called singlet oxygen ($^1\text{O}_2^*$). Carotenoids prevent this by quenching the excited state of chlorophyll.

(iv) Three xanthophylls (violoxanthin, antheroxanthin and zeaxanthin) take part in dissipation of excess energy by conversion of the same into heat.

(v) By their colour, the carotenoids make the flowers and fruits conspicuous to animals for pollination and dispersal.

(vi) β -carotene produces vitamin A in animals.

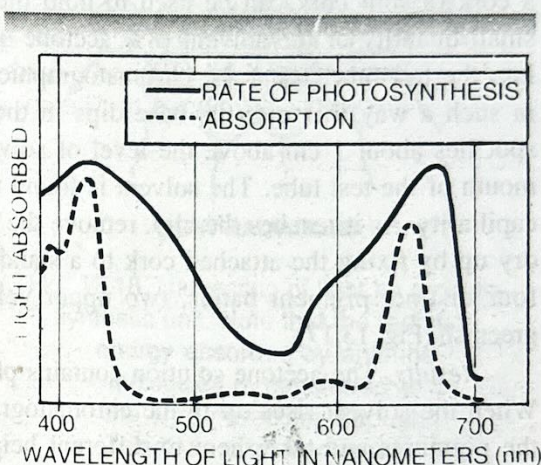


Fig. 13.16. Action spectrum of photosynthesis and absorption spectrum of chlorophyll *a*.

Expt. 5. Demonstration and Separation of Photosynthetic Pigments.

Apparatus. Fresh green leaves, acetone, petroleum ether, fine sand, pestle and mortar, a narrow gas jar or a large test tube, test tube stand, dropper, chromatographic paper or Whatman filter paper, paper clip, filtering apparatus, water bath, beaker, measuring cylinder, scissors.

Working. Take a few fresh green leaves in a mortar. Add a small quantity of fine washed sand and 15 ml of acetone. Grind well with the help of pestle and filter the pulp. The filtrate is evaporated over a water bath (40°–50°C) till a small quantity of it is left. It is the pigment concentrate.

Cut a strip of chromatographic paper, narrower than the diameter of a test tube or gas jar. Produce two lateral notches about 2–3 cm from one end. Place a few drops of pigment concentrate one by one on the same spot in the notched part of chromatographic strip and allow it to dry. Connect the other end of the strip with a clip fitted in a cork (a split cork can be used to hold the strip directly). Pour a small quantity of the solvent (8% acetone + 92% petroleum ether) into the test tube. Hang the chromatographic strip into the test tube in such a way that only its base dips in the solvent. The pigment spot lies about 1 cm above the level of solvent. Fit the cork in the mouth of the test tube. The solvent is found to move up the strip by capillarity. As it reaches the clip, remove the strip. Allow the strip to dry up by fixing the attached cork to a stand. The dried strip shows four distinct pigment bands, two upper yellowish and two lower greenish (Fig. 13.17).

Results. The acetone solution contains photosynthetic pigments. When the solvent rises up in the chromatographic strip, it dissolves the pigments and take them to different heights according to their solubility. The occurrence of four pigment bands shows that photosynthetic pigments are of four types, two yellowish carotenoids and two greenish chlorophylls. The four bands starting from above are orange yellow of carotenes, yellowish of xanthophylls, bluish green of chlorophyll *a* and yellowish green of chlorophyll *b*.

Precautions. (i) Take only a few small flush green fresh leaves. (ii) Evaporate the pigment solution slowly. (iii) Do not allow the chromatographic strip to touch the walls of the test tube. (iv) The pigment concentrate should be loaded carefully. It should not spread on the strip. (v) The pigment spot should not dip in the solvent. (vi) The cork should be air tight.

Phycobilins. They are open tetrapyrroles which neither contain magnesium nor phytol. Phycobilins are water soluble. However, they occur in association with proteins or biliproteins. The pigments are of two types—blue (phycocyanin, allo-phycocyanin) and red (phycoerythrin). The pigments are useful in **chromatic adaptations**. They are important accessory pigments of blue-green algae, cryptomonads and red algae. In blue-green and red algae, the phycobilins are found inside submicroscopic structures called phycobilisomes attached to thylakoids.

Photosynthetic Units (PSU, Fig. 13.18)

A photosynthetic unit is the smallest group of pigment molecules which take part in a photochemical act or conversion of light energy into chemical energy. It has a **photochemical reaction centre** which is fed by about 200 **harvesting pigment molecules**. The photochemical reaction centre consists of a **dimer** (Taiz and Zeiger, 2002) of special chlorophyll *a* molecules, P_{700} or P_{680} (named after maximum absorption by pigment or photochemical reaction centre). Reaction centre absorbs light energy at longer wavelengths. The harvesting molecules form a protein based complex called

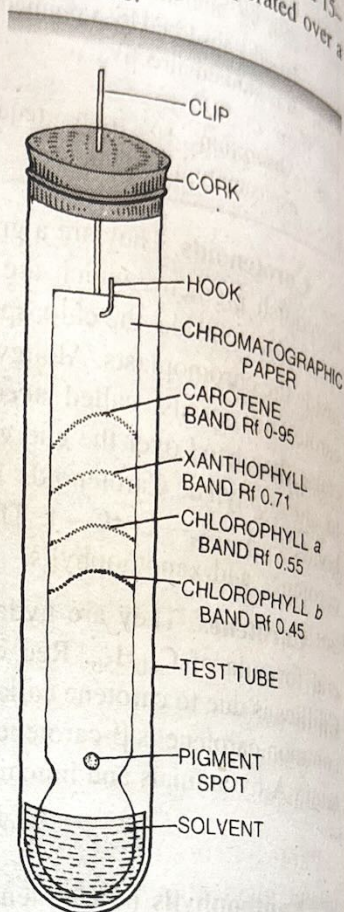


Fig. 13.17. Separation of photosynthetic pigments by Simple Paper Chromatography.

light harvesting complex (LHC). There are distinct LHCs for PS I and PS II. Light harvesting molecules are of two types, **antenna molecules** and **core molecules**. The antenna molecules absorb light of various wavelengths but shorter than that of photocentre. On absorption of light energy the antenna molecules get excited. In the **excited state** an electron is pushed to an outer orbital. It lasts for about 10^{-9} seconds. The excited antenna molecules hand over their energy to core molecules by resonance and come to the **ground state**. The energy picked up by core molecules is supplied to the trap or photocentre. On absorption of energy the photocentre gets excited and extrudes an electron after which it comes to ground state to repeat the cycle. The frequency of excitation of photocentre is very high. It cannot be met by its direct absorption of sun energy. Moreover, the absorption of light of shorter wavelengths cannot be done by it directly. Therefore, photocentre requires the help of harvesting molecules in the absorption of light energy. Another requirement is the regular supply of electrons from another system.

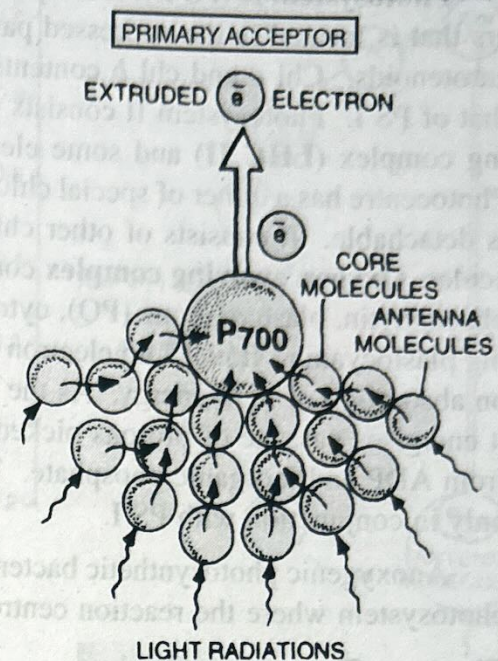


Fig. 13.18. Harvesting of light by a photosynthetic unit. Note that the radiation energy absorbed by antenna molecules is transferred to the core molecules through electron spin resonance.

Photosystems or Pigment Systems (Fig. 13.19)

In green plants, photosynthetic units occur in the form of two distinct groups called photosystems or pigment systems, I and II. They are named after the sequence they were discovered. Each photosystem contains 250–400 pigment molecules.

Photosystem I (PS I). It is a photosynthetic pigment system along with some electron carriers that is located on both the nonappressed part of grana thylakoids as well as stroma thylakoids. PS I has more of chlorophyll *a*. Chlorophyll *b* and carotenoids are comparatively less. Photosystem I consists of a photocentre, light harvesting complex (LHC I) and some electron carriers. All are based over membrane proteins. Photocentre has a dimer of special chlorophyll *a* molecules called P_{700} . Light harvesting complex has other chlorophyll *a* molecules, followed by chlorophyll *b* and carotenoids. Photosystem I has a reducing agent A_0 (which is a special chlorophyll P_{700} molecule), A_1 (a quinone), $Fe S_X$, $Fe S_A$ and $Fe S_B$ (iron-sulphur proteins), Fd (ferredoxin), cytochrome b_6-f , complex and plastocyanin. It takes part in both cyclic and noncyclic photophosphorylation. PS I can carry on cyclic photophosphorylation independently. Normally it drives an electron from photosystem II to $NADP^+$ as component of light reaction.

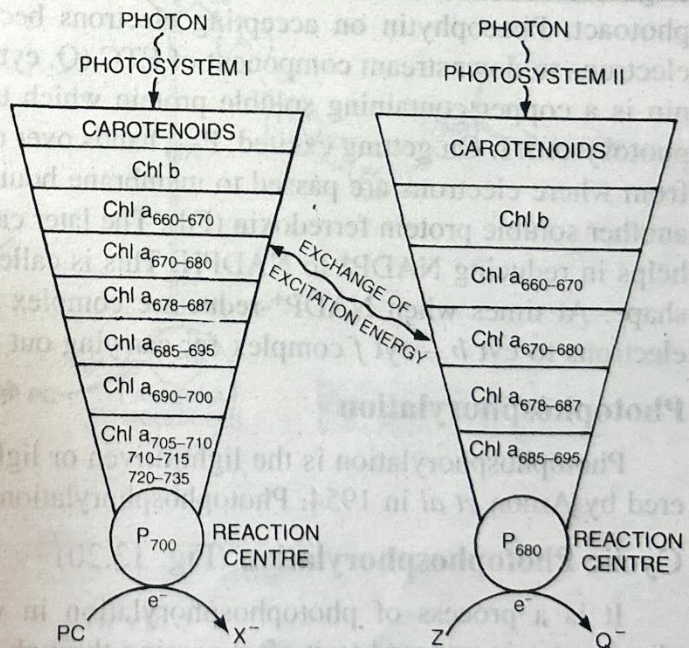


Fig. 13.19. Distribution of pigments in the two photosystems or pigment systems.

Photosystem II (PS II). It is a photosynthetic pigment system along with some electron carriers that is located in the appressed part of the grana thylakoids. PS II has chlorophyll *a*, *b* and carotenoids. Chl *a* and chl *b* contents are equal. Carotenoid content is higher as compared to that of PS I. Photosystem II consists of a photocentre, oxygen evolving complex, light harvesting complex (**LHC II**) and some electron carriers. All are connected to membrane proteins. Photocentre has a dimer of special chlorophyll *a* molecules called P_{680} . Light harvesting complex is detachable. It consists of other chlorophyll *a* molecules, chlorophyll *b* and carotenoid molecules. **Oxygen evolving complex** contains Mn^{2+} , Ca^{2+} and Cl^{-} . Other components of PS II are phaeophytin, plastoquinone (PQ), cytochrome $b_6 - f$ complex and blue coloured copper containing plastocyanin. It picks up electron released during photolysis of water. The same is extruded on absorption of light energy. As the extruded electron passes over cytochrome $b_6 - f$ complex, it energises passage of protons picked up by PQ to create proton gradient for synthesis of ATP from ADP and inorganic phosphate. This photophosphorylation is noncyclic. PS II can operate only in conjunction with PS I.

Anoxygenic photosynthetic bacteria (e.g., *Rhodobacter*, *Rhodospseudomonas*) possess a single photosystem where the reaction centre is similar to that of photosystem II.

Electron Transport Chain

It was first formulated by Hill (1939) while details were worked out later on. Electron transport chain is a series of electron carriers over which electrons pass in a downhill journey releasing energy at every step that is used in generating an electro chemical proton gradient which helps in synthesising ATP. Photosynthetic electron transport chain has two components connected with the two photosystems. P_{680} of photosystem II absorbs light energy, gets excited and transfers its electrons to electron acceptor molecule phaeophytin. After losing electrons, P_{680} becomes a strong oxidant, paves the way for light dependent splitting of water called **photolysis**. It generates electrons which are passed on to electron deficient P_{680} for performing another photoact. Phaeophytin on accepting electrons becomes strong reducing agent. It donates its electrons to downstream component of ETC (Q, cyt *b* - cyt *f* complex, plastocyanin). Plastocyanin is a copper containing soluble protein which transfers electrons to P_{700} reaction centre of photosystem I. On getting excited, P_{700} hands over electrons to a special chlorophyll molecule X from where electrons are passed to membrane bound iron sulphur proteins (FeS) for transfer to another soluble protein ferredoxin (Fd). The later can pass electrons to reductase complex which helps in reducing $NADP^{+}$ to NADPH. This is called **Z scheme** due to its characteristic zig zag shape. At times when $NADP^{+}$ -reductase complex is not operating, ferredoxin can pass on its electrons to cyt *b* - cyt *f* complex for carrying out cyclic photophosphorylation.

Photophosphorylation

Photophosphorylation is the light driven or light energised synthesis of ATP. It was discovered by Arnon *et al* in 1954. Photophosphorylation is of two main types, cyclic and noncyclic.

Cyclic Photophosphorylation (Fig. 13.20)

It is a process of photophosphorylation in which an electron expelled by the excited photocentre is returned to it after passing through a series of electron carriers. It occurs under conditions of low light intensity, wavelength longer than 680 nm and when CO_2 fixation is inhibited. Absence of CO_2 fixation results in nonrequirement of electrons for formation of NADPH. Cyclic photophosphorylation is performed by photosystem I only. Its photocentre P_{700} extrudes an electron with a gain of 23 kcal/mole of energy after absorbing a photon of light ($h\nu$). After losing the electron the photocentre becomes oxidised. The expelled electron passes through a

series of carriers including X or A_0 (a special P_{700} chlorophyll molecule), A_1 (a quinone), FeS complexes (FeS_X , FeS_A , FeS_B), ferredoxin, (Fd), plastoquinone (PQ), cytochrome $b-f$ complex and plastocyanin before returning to photocentre. While over the cytochrome complex, the electron energises passage of protons to create a proton gradient for synthesis of ATP from ADP and inorganic phosphate.

Halobacteria or halophile bacteria also perform photophosphorylation but ATP thus produced is not used in synthesis of food. These bacteria possess purple pigment bacteriorhodopsin attached to plasma membrane. As light falls on the pigment, it creates a proton pump which is used in ATP synthesis.

Noncyclic Photophosphorylation (Fig. 13.21)

It is the normal process of photophosphorylation in which the electron expelled by the excited photocentre does not return to it. Non-cyclic photophosphorylation is carried out in collaboration of both photosystems I and II. Electron released during photolysis of water is picked up by photocentre of PS II called P_{680} . The same is extruded out when the photocentre absorbs light energy (hv).

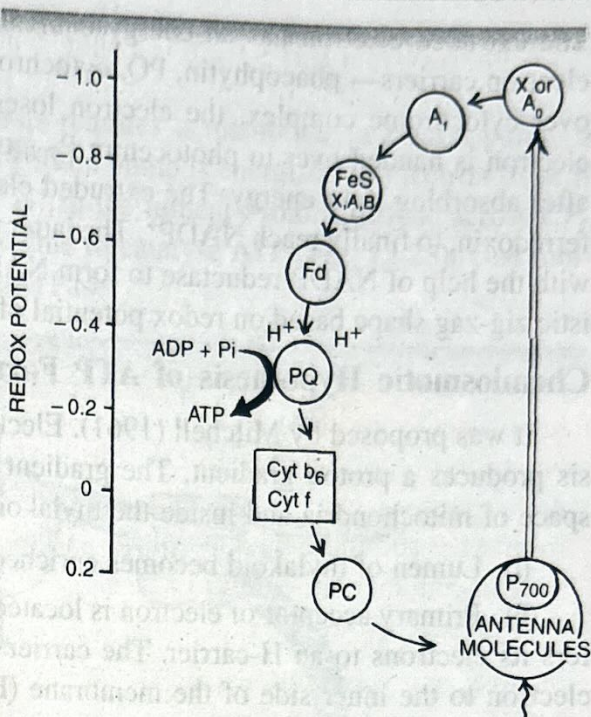


Fig. 13.20. Cyclic photophosphorylation.

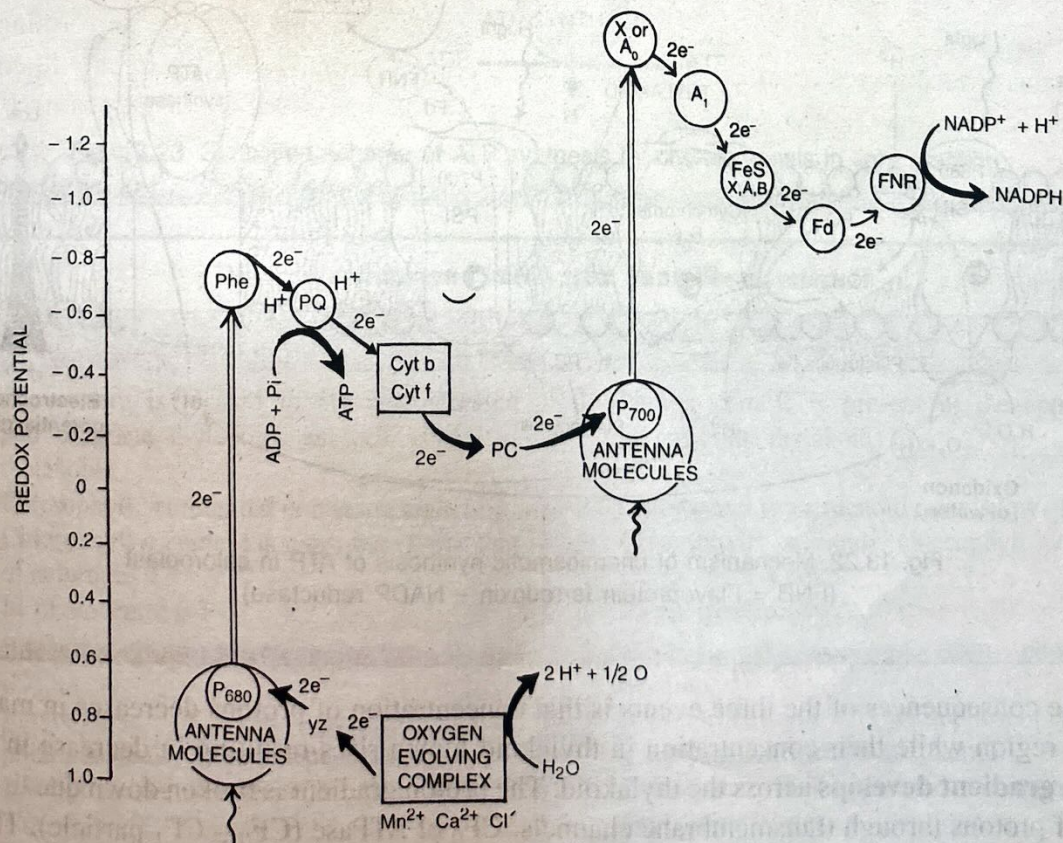


Fig. 13.21. Non-cyclic photophosphorylation and electron transport during photochemical phase.

The extruded electron has an energy equivalent to 23 kcal/mole. It passes through a series of electron carriers—phaeophytin, PQ, cytochrome *b-f* complex and plastocyanin. While passing over cytochrome complex, the electron loses sufficient energy for the synthesis of ATP. The electron is handed over to photocentre P_{700} of PS I by plastocyanin. P_{700} extrudes the electron after absorbing light energy. The extruded electron passes through special chlorophyll X, Fe-S, ferredoxin, to finally reach $NADP^+$. The latter then combines with H^+ (released during photolysis) with the help of NADP-reductase to form NADPH. This is called **Z scheme** due to its characteristic zig-zag shape based on redox potential of different electron carriers (Fig. 13.21).

Chemiosmotic Hypothesis of ATP Formation

It was proposed by Mitchell (1961). Electron transport, both in respiration and photosynthesis produces a proton gradient. The gradient develops in the outer chamber or intermembrane space of mitochondria and inside the thylakoid lumen in chloroplasts.

(i) Lumen of thylakoid becomes enriched with H^+ ion due to photolytic splitting of water.
 (ii) Primary acceptor of electron is located on the outer side of thylakoid membrane. It transfers its electrons to an H-carrier. The carrier removes a proton from matrix while transporting electron to the inner side of the membrane (Fig. 13.22). The proton is released into the lumen while the electron passes to the next carrier.

(iii) NADP reductase is situated on the outerside of thylakoid membrane. It obtains electron from PS I and protons from matrix to reduce $NADP^+$ to $NADP + H^+$ state.

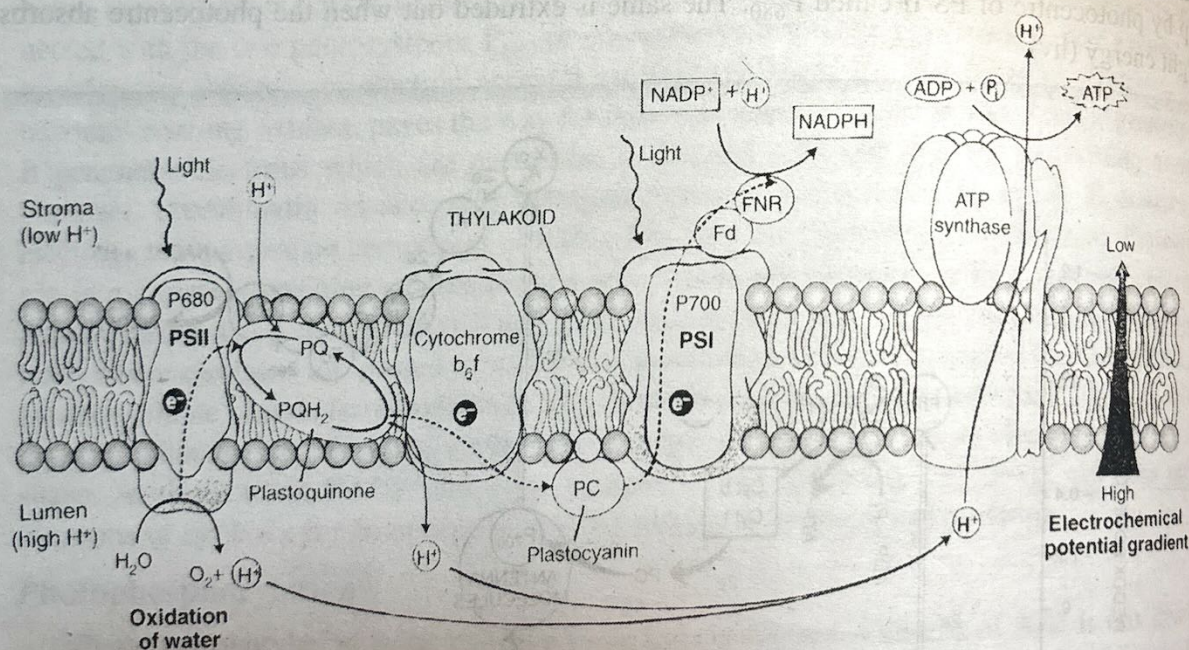


Fig. 13.22. Mechanism of chemiosmotic synthesis of ATP in chloroplast (FNR = Flavoprotein ferredoxin – NADP reductase).

The consequences of the three events is that concentration of protons decreases in matrix or stroma region while their concentration in thylakoid lumen rises resulting in decrease in pH. A **proton gradient develops** across the thylakoid. The proton gradient is broken down due to movement of protons through transmembrane channels, CF_0 of ATPase ($CF_0 - CF_1$ particle). The rest of the membrane is impermeable to H^+ . [CF_0 provides facilitated diffusion to H^+ or protons.] As protons move to the other side of ATP, they bring about conformational changes in CF_1 particle

of ATPase or coupling factor. The transient CF_1 particle of ATPase enzyme form ATP from ADP and inorganic phosphate.

Therefore, ATP synthesis through chemiosmosis requires a membrane, a proton pump, a proton gradient and $CF_0 - CF_1$ particle or ATP-ase. Proton pump is energised by electron flow. It creates a proton gradient or high concentration of H^+ in the lumen. Protons diffuse across CF_0 channels, release energy that activates ATP-ase enzyme to catalyse ATP (Fig. 13.23). One molecule of ATP is formed when $2 H^+$ pass through ATPase.

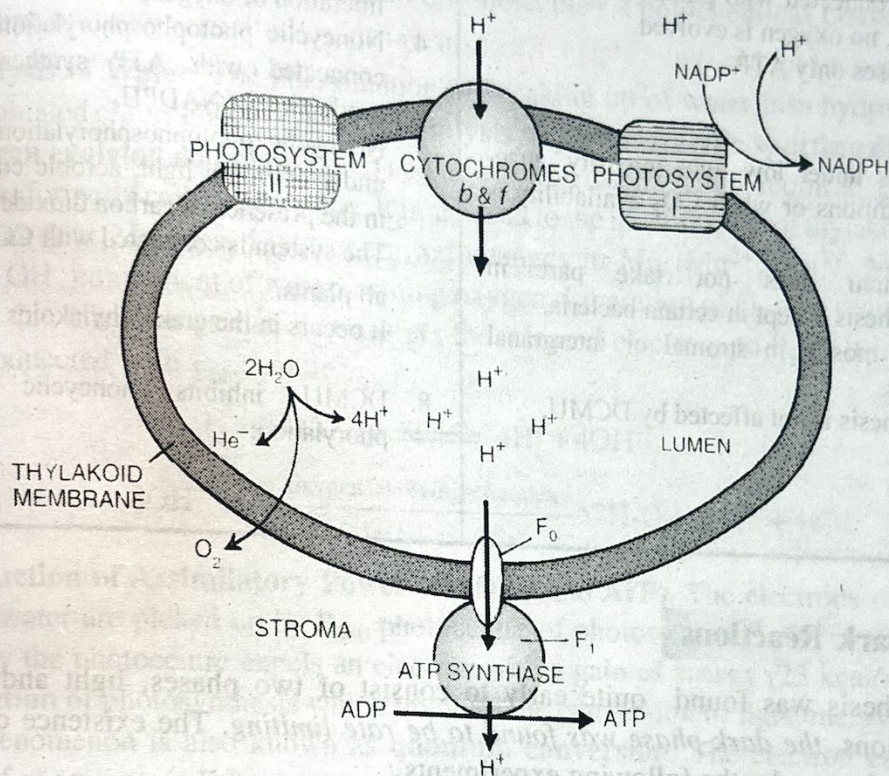


Fig. 13.23. Simplified scheme of ATP synthesis by chemiosmosis in light reaction.

Differences between Photosystem I and Photosystem II

Photosystem I	Photosystem II
1. The system is located in the nonappressed part of grana thylakoids as well as stroma thylakoids.	1. Photosystem II is present in the appressed part of grana thylakoids.
2. Chlorophyll : carotenoid content is high.	2. Chlorophyll to carotenoid ratio is low.
3. Chlorophyll <i>a</i> content is more than twice that of chlorophyll <i>b</i> .	3. Chlorophyll <i>a</i> and chlorophyll <i>b</i> are approximately equal.
4. Its photocentre is P_{700} .	4. Its photocentre is P_{680} .
5. It receives electrons from photosystem II.	5. Electrons are received from photolytic reaction.
6. Photosystem I can perform cyclic photophosphorylation independently.	6. It performs non-cyclic photophosphorylation in conjunction with photosystem I.
7. It is not connected with photolysis of water.	7. Photosystem II is connected with photolytic oxidation of water.
8. Usually it hands over its electron to $NADP^+$.	8. Usually it hands over its electrons to PS I.

* Now $3H^+$ are thought to help form one ATP.

Differences between Cyclic and Non-cyclic Photophosphorylation

Cyclic Photophosphorylation	Non-cyclic Photophosphorylation
1. It is performed by photosystem - I independently. 2. An external source of electrons is not required because the same electrons get recycled. 3. It is not connected with photolysis of water. Therefore, no oxygen is evolved. 4. It synthesises only ATP. 5. It operates under low light intensity, anaerobic conditions or when CO₂ availability is poor. 6. The system does not take part in photosynthesis except in certain bacteria. 7. It occurs mostly in stromal or intergranal thylakoids. 8. ATP synthesis is not affected by DCMU.	1. It is performed by collaboration of both photosystems II and I. 2. The process requires an external electron donor. 3. It is connected with photolysis of water and liberation of oxygen. 4. Noncyclic photophosphorylation is not only connected with ATP synthesis but also production of NADPH. 5. Noncyclic photophosphorylation takes place under optimum light, aerobic conditions and in the presence of carbon dioxide. 6. The system is connected with CO₂ fixation in all plants. 7. It occurs in the granal thylakoids. 8. DCMU inhibits noncyclic photophosphorylation.

Light and Dark Reactions

Photosynthesis was found quite early to consist of two phases, light and dark. Under normal conditions, *the dark phase was found to be rate limiting*. The existence of two phases has come to be known by the following experiments :

1. **Flashing Light Experiments.** Warburg (1919) obtained higher rates of photosynthesis in *Chlorella* when it was exposed to rapid and alternate periods of light and darkness instead of continuous illumination. Emerson and Arnold (1932) found that at 25°C, the maximum photosynthesis took place when light and dark periods were respectively 10⁻⁵ sec and 0.055 sec. At 1.1°C, maximum photosynthesis could be obtained with the same light period but the dark period had to be increased to 0.4 sec. It means that temperature influences reactions of only the dark period which must be purely chemical. Reactions of the light phase are photochemical.

2. **Temperature Effects.** At low light intensity, increase in temperature does not increase the rate of photosynthesis. At optimum light intensity and CO₂ availability, rate of photosynthesis can be increased or decreased with increase or decrease in temperature.

3. **Inhibitors.** Warburg (1920) applied the low concentration of cyanide to the photosynthetic regions. It inhibited photosynthesis under high light intensity more than that at low light intensity.

4. **Induction Phase.** When a green plant is suddenly illuminated, there is a gush of oxygen production without simultaneous absorption of CO₂.

5. **Dark Pick up of Carbon Dioxide.** Carbon dioxide can be fixed in the dark by previously illuminated green cells.

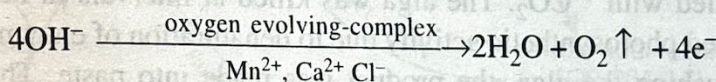
Mechanism of Photosynthesis

Photosynthesis occurs in two phases— **photochemical** and **biosynthetic**. Photochemical phase is also called **light** or **Hill reaction** (after the name of the scientist who discovered its details). Biosynthetic phase is also termed as **dark** or **Blackman's** (after the name of scientist who first postulated it) **reaction**.

Photochemical Phase (Light or Hill Reaction, Fig. 13.21)

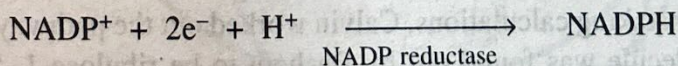
It occurs inside the thylakoids, especially those of grana region. Photochemical step is dependent upon light. The function of this phase is to produce **assimilatory power** consisting of reduced coenzyme NADPH and energy rich ATP molecules. Photochemical phase involves the following reactions :

(a) **Photolysis of Water**. The phenomenon of breaking up of water into hydrogen and oxygen in the illuminated chloroplasts is called **photolysis** or **photocatalytic splitting of water**. Light energy, an **oxygen evolving complex** (OEC) and an electron carrier Y_z are required. Oxygen evolving complex was formerly called **Z-enzyme**. It is attached to the inner surface of thylakoid membrane. The enzyme has four Mn ions. Light energised changes in Mn (Mn^{2+} , Mn^{3+} , Mn^{4+}) removes electrons from OH^- component of water forming oxygen. Liberation of O_2 also requires two other ions, Ca^{2+} and Cl^- . Electron carrier Y_z transfers the released electrons to P_{680} (It is a component of a protein connected with P_{680} centre).



(b) **Production of Assimilatory Power** (NADPH and ATP). The electrons released during photolysis of water are picked up by P_{680} photocentre of photosystem II. On receiving a photon of light energy the photocentre expels an electron with a gain of energy (23 kcal/mole). It is the **primary reaction** of photosynthesis which involves the conversion of light energy into chemical form. The phenomenon is also known as **quantum conversion**. The electron extruded by the photocentre of photosystem II is picked up by the quencher phaeophytin. From here the electron passes over a series of carriers in a downhill journey losing its energy at every step. The major carriers are plastoquinone (PQ), cytochrome $b-f$ complex and plastocyanin (PC). While passing over cytochrome complex, the electron loses sufficient energy for the creation of proton gradient and synthesis of ATP from ADP and inorganic phosphate. The process is called **photophosphorylation** (noncyclic).

From plastocyanin the electron is picked up by the trap centre P_{700} of photosystem I. On absorbing a photon of light energy, P_{700} pushes out the electron with a gain of energy. The electron passes over carriers X (a special chlorophyll molecule), FeS, ferredoxin and NADP-reductase. The latter gives electrons to $NADP^+$ for combining with H^+ ions to produce NADPH.



NADPH is a strong reducing agent. It constitutes the **reducing power** which also contains a large amount of chemical energy.

Biosynthetic Phase (Dark or Blackman's Reaction)

It catalyses assimilation of CO_2 to carbohydrates. The reactions are called **carbon reactions**. They occur in stroma or matrix of chloroplasts. The reactions do not require light. Instead assimilatory power (ATP and NADPH) produced during photochemical phase is used in fixation

and reduction of carbon dioxide. All the enzymes required for the process are present in the matrix or stroma of the chloroplast. There are two main pathways for the biosynthetic or dark phase—Calvin cycle and C_4 dicarboxylic acid cycle. A third pathway is CAM metabolism which is intermediate between the two. The plants exhibiting Calvin cycle and C_4 dicarboxylic acid cycle are respectively called C_3 and C_4 plants. There is no taxonomic importance of the two pathways. Thus *Euphorbia corollata* is a C_3 plant while *Euphorbia maculata* is C_4 plant. Grass *Alloteropsis semi-alata* has both C_3 and C_4 ecotypes (ecological variants).

Differences between Photochemical and Biosynthetic Phases	
Photochemical Phase	Biosynthetic Phase
1. It occurs over thylakoids.	1. It occurs in matrix of chloroplasts.
2. The phase is dependent on light.	2. The phase does not require light.
3. Temperature has little effect over it.	3. It is influenced by temperature.
4. It produces NADPH and ATP (assimilatory power).	4. It consumes NADPH and ATP.
5. Oxygen is evolved.	5. Glucose is end product.

Calvin Cycle (Calvin, 1955; Calvin-Benson Cycle, Reductive Pentose Phosphate Pathway or RPP, C_3 Cycle)

History. The cycle was discovered by Calvin, Benson and their colleagues in California, U.S.A. They fed *Chlorella* and *Scenedesmus* with radioactive ^{14}C in carbon dioxide. Radioactive carbon, ^{14}C has a half life of 5568 years. Therefore, the path of CO_2 fixation can be easily traced with its help. Algal suspension illuminated and carrying out photosynthesis with normal carbon dioxide was supplied with $^{14}CO_2$. The alga was killed at intervals in near boiling methanol. It immediately stopped photosynthetic activity due to denaturation of enzymes. Alcohol was evaporated and after crushing the alga, the product was made into paste. The paste was placed on paper chromatogram and the different compounds were separated by two dimensional chromatography first developed by Martin and Synge (1941). An X-ray film was then pressed against the paper chromatogram. The film developed spots where radioactive compounds were present. The process is called **autoradiography**. The radioactive compounds were identified by comparing their position on the chromatogram with standard chemicals.

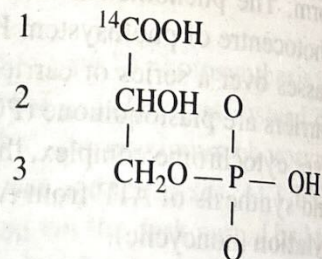
Calvin and co-workers found that after three seconds, radioactivity appeared in phosphoglyceric acid or PGA. Phosphoglyceric acid is, therefore, the first stable product of photosynthesis. Radioactivity was also found out to be present in only one carbon of this compound which happened to be the first one.

Apparently only the first carbon group of the chemical came from CO_2 while the rest were contributed by some acceptor molecule. A number of other compounds having radioactivity were found after intervals of 5, 10, 15 and 30 seconds. They included hexoses, tetroses, pentoses, heptoses. After 60 seconds, all the three carbon atoms of PGA were radioactive indicating cycling of reactions. After many painstaking calculations, Calvin worked out the pathway of CO_2 fixation. The **primary acceptor** molecule was found out by Basham to be ribulose-1, 5-bisphosphate or RuBP.

Phases of Calvin Cycle (Calvin, 1955)

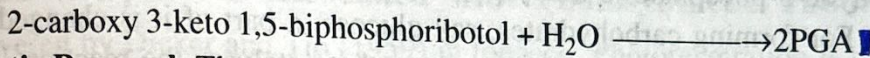
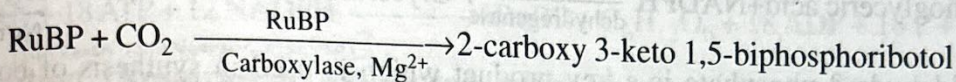
Photosynthetic Carbon Reduction (PCR) Cycle or Calvin cycle occurs in all photosynthetic plants whether they have C_3 or C_4 pathways. It is divided into the following three phases—carboxylation, glycolytic reversal and regeneration of RuBP (Fig. 13.24).

1. **Carboxylation.** Carboxylation is the addition of carbon dioxide to another substance



called acceptor. Photosynthetic carboxylation requires ribulose-1, 5-biphosphate or RuBP as **acceptor** of carbon dioxide and **RuBP carboxylase-oxygenase** or **RuBisCo** as enzyme. The enzyme was previously called **carboxydismutase**. Rubisco is the most abundant protein of the biological world. It constitutes 16% of chloroplast proteins (40% of soluble leaf proteins). However, it is a slowest enzyme with a turnover of 3 carbon dioxide molecules per second. Rubisco is located in the stroma on the outer surface of thylakoid membranes.

Carbon dioxide combines with ribulose-1, 5-biophosphate to produce a transient intermediate compound called 2-carboxy 3-keto 1,5-biphosphoribitol. The intermediate splits up immediately in the presence of water to form the two molecules of **3-phosphoglyceric acid** or PGA. It is the first stable product of photosynthesis.



2. **Glycolytic Reversal.** The processes involved in this step or phase are reversal of the

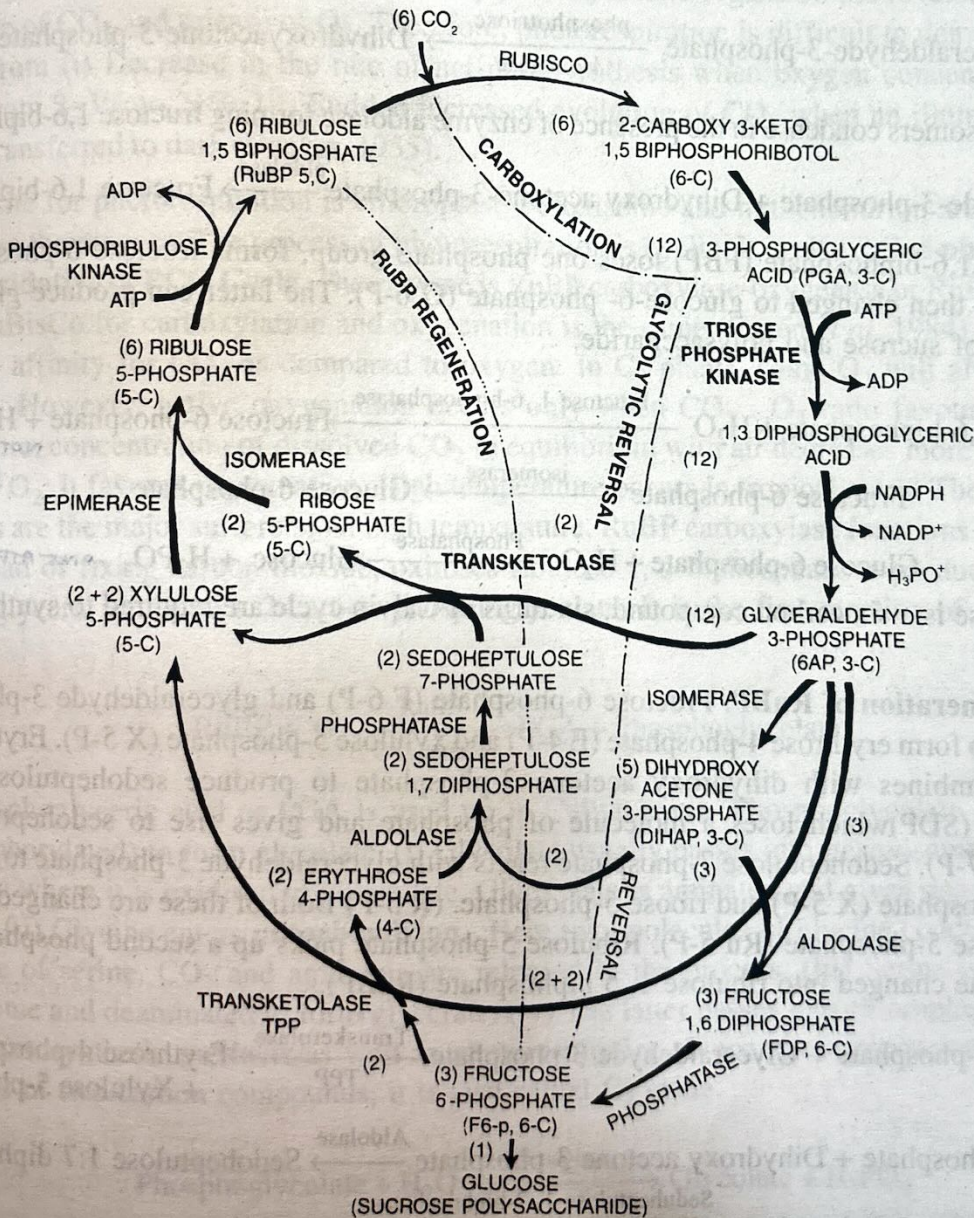
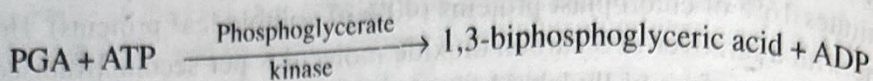
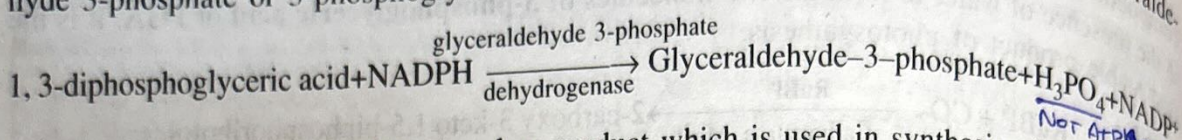


Fig. 13.24. Calvin cycle.

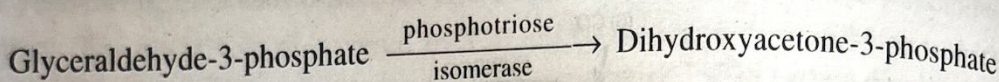
processes found during glycolysis part of respiration. Phosphoglyceric acid or PGA is further phosphorylated by ATP with the help of enzyme triose phosphate kinase (phosphoglycerate kinase). It gives rise to 1, 3-biphosphoglyceric acid.



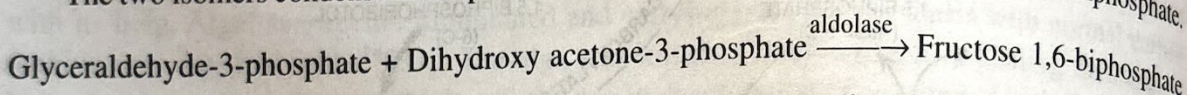
Biphosphoglyceric acid is reduced by NADPH through the agency of enzyme glyceraldehyde 3-phosphate dehydrogenase (triose phosphate dehydrogenase). It produces glyceraldehyde 3-phosphate or 3-phosphoglyceraldehyde.



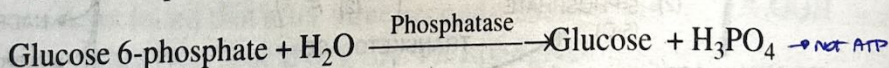
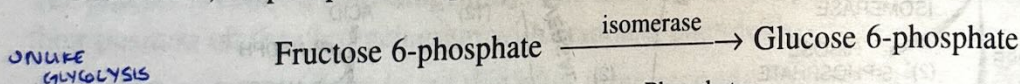
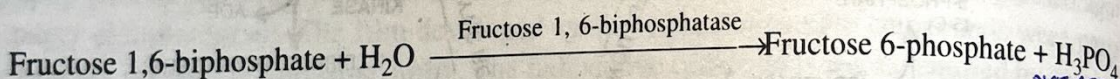
Glyceraldehyde-3-phosphate is a key product which is used in synthesis of both carbohydrates and fats. For forming carbohydrates, say glucose, a part of it is changed into its isomer called dihydroxyacetone-3-phosphate. The enzyme that catalyses the reaction is phosphotriose isomerase.



The two isomers condense in the presence of enzyme aldolase forming fructose 1,6-biphosphate.

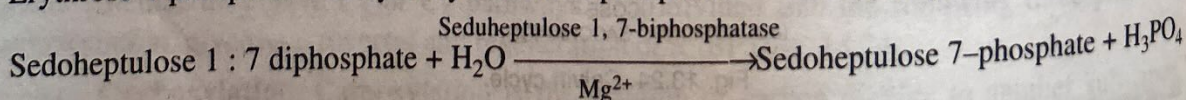
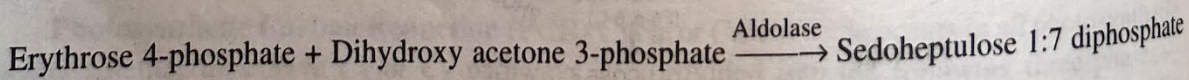
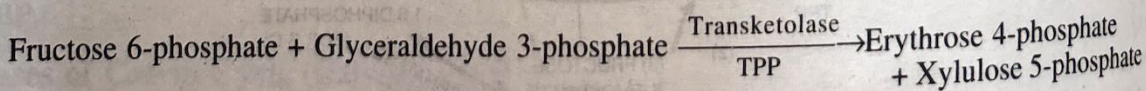


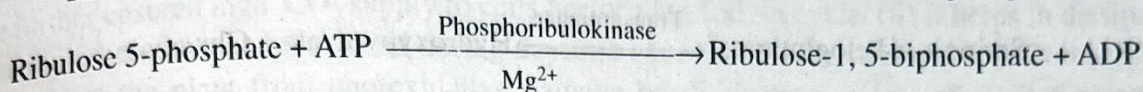
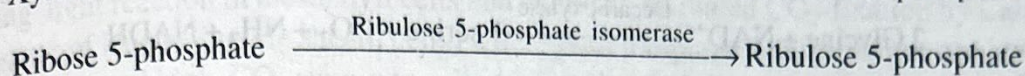
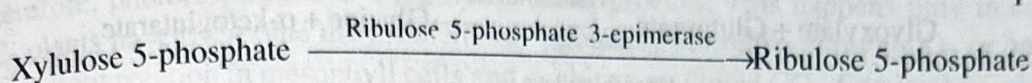
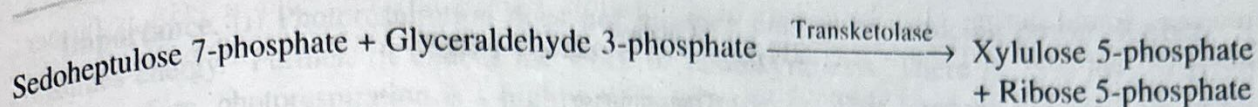
Fructose 1,6-biphosphate (FBP) loses one phosphate group, forms fructose 6-phosphate (F 6-P) which is then changed to glucose-6-phosphate (G 6-P). The latter can produce glucose or become part of sucrose and polysaccharide.



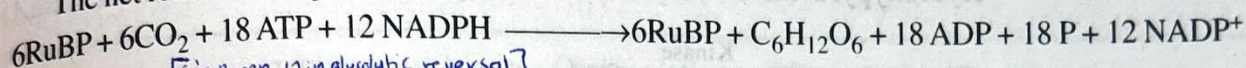
As glucose is a six carbon compound, six turns of Calvin cycle are required to synthesise its one molecule.

3. Regeneration of RuBP. Fructose 6-phosphate (F 6-P) and glyceraldehyde 3-phosphate (GAP) react to form erythrose 4-phosphate (E 4-P) and xylulose 5-phosphate (X 5-P). Erythrose 4-phosphate combines with dihydroxy acetone 3-phosphate to produce sedoheptulose 1 : 7 diphosphate (SDP) which loses a molecule of phosphate and gives rise to sedoheptulose 7-phosphate (S 7-P). Sedoheptulose 7-phosphate reacts with glyceraldehyde 3-phosphate to produce xylulose 5-phosphate (X 5-P) and ribose 5-phosphate. (R 5-P) Both of these are changed to their isomer ribulose 5-phosphate (Ru 5-P). Ribulose 5-phosphate picks up a second phosphate from ATP to become changed into ribulose 1, 5 biphosphate (RuBP).





The net reaction of C_3 dark fixation of carbon dioxide is

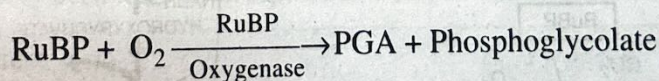


[in Regen 12:inglydytic reversal]

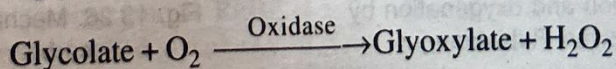
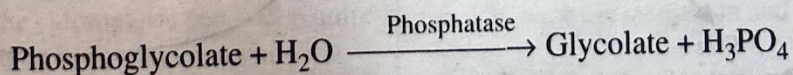
Photorespiration (Respiration Associated with Photosynthetic Tissues)

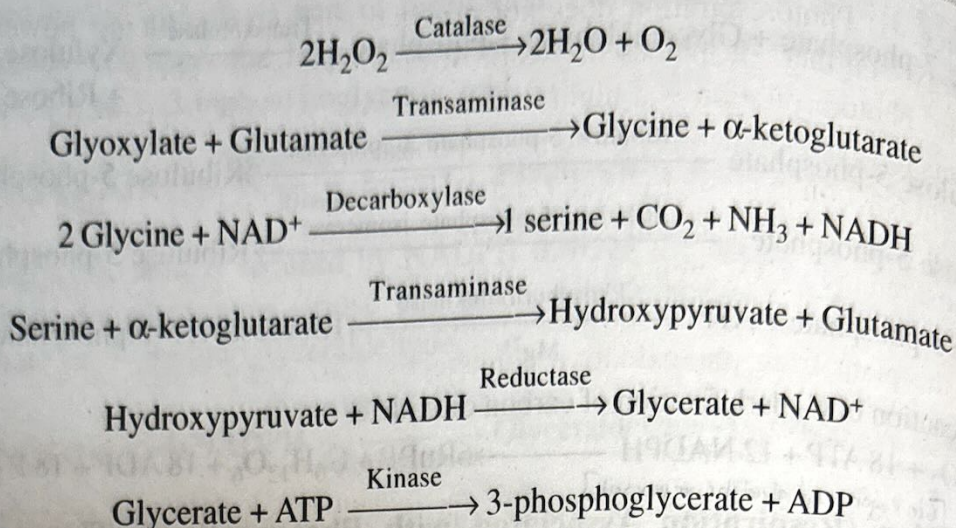
It was discovered by Dicker and Tio (1959) in tobacco. Photorespiration is the light dependent process of oxygenation of ribulose biphosphate (RuBP) and release of carbon dioxide by the photosynthetic organs of a plant. Normally photosynthetic organs do the reverse in the light, i.e., uptake of CO_2 and release of O_2 . Therefore, photorespiration is difficult to demonstrate. It is inferred from (i) Decrease in the rate of net photosynthesis when oxygen concentration is increased from 2–3% to 21%. (ii) Sudden increased evolution of CO_2 when an illuminated green organ is transferred to dark (Decker, 1955).

The site for photorespiration is chloroplast. Peroxisome and mitochondrion are required for completing the process. The process of photorespiration is cyclic. It is also called **photosynthetic carbon oxidative** or **PCO Cycle**. The enzyme is RuBP carboxylase-oxygenase or RuBisCo. Active site of RuBisCo for carboxylation and oxygenation is the same (Ogren *et al*, 1984). The enzyme has more affinity for CO_2 as compared to oxygen. In C_3 plants, some O_2 will always bind to RuBisCo. However, active oxygenation occurs only when $\text{CO}_2 : \text{O}_2$ ratio favours it. At high temperature, concentration of dissolved CO_2 in equilibrium with air decreases more than concentration of O_2 . It favours oxygenation. High temperature occurs in tropical areas. Therefore, tropical plants are the major sufferers. At high temperature, RuBP carboxylase functions as oxygenase and instead of fixing carbon dioxide, oxidises ribulose 1, 5-biphosphate to produce a 3-carbon phosphoglyceric acid and a 2-carbon phosphoglycolate. It is the first reaction of photorespiration.



Phosphoglyceric acid or PGA is used up in Calvin cycle. Phosphoglycolate is hydrolysed (dephosphorylated) to form glycolate. (i) Glycolate usually passes into **peroxisome** of the mesophyll cell where it is oxidised to glyoxylate. Glyoxylate is aminated and gives rise to amino acid glycine. (ii) Glycine enters **mitochondrion**. Here two molecules of glycine condense to form a molecule of serine. CO_2 and ammonia are released in the process. (iii) Serine is taken up by peroxisome and deaminated to form glycerate. (iv) The latter passes into chloroplasts for synthesis of photosynthetic products as well as photorespiration. Since photorespiration involves the synthesis of two-carbon compounds, it is also called **C_2 cycle**.





In photorespiration two molecules of phosphoglycolate formed by oxygenation of RuBP is changed into 1 molecule of phosphoglycerate (PGA) and one molecule of CO_2 . In other words 75% of carbon lost during oxygenation of RuBP is recovered by photorespiratory carbon oxygenation or PCO cycle.

Photorespiration increases with increase of (i) Intensity of light, (ii) Concentration of oxygen, (iii) Temperature and (iv) Age of the leaf. However, C_4 plants are an exception. They are specialised tropical plants which ensure that photorespiration does not occur in them. RuBisCo is located only in bundle sheath cells where photosynthetic release of oxygen does not occur. Bundle sheath cells have a high intracellular concentration of CO_2 due to flow of C_4 acids and their decarboxylation to release CO_2 . Therefore, RuBisCo functions purely as carboxylase in C_4 plants. C_4 plants also have a high tolerance to high temperature, aridity and salinity. They show high productivity and hence higher yield.

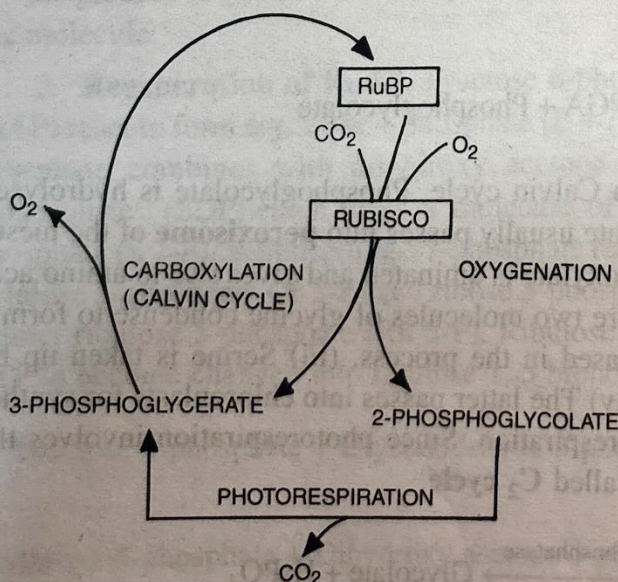


Fig. 13.25. Carboxylation and oxygenation by the same enzyme Rubisco.

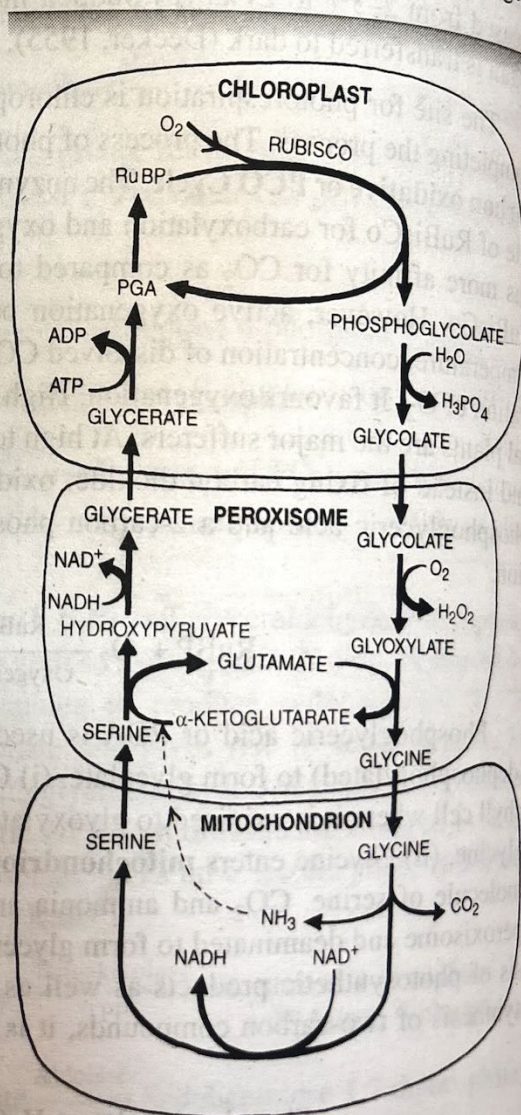


Fig. 13.26. Mechanism of photorespiratory carbon oxidation cycle.

Importance. (i) Photorespiration does not produce energy or reducing power. Rather, it consumes energy. Further, it undoes the work of photosynthesis. There is 25% loss of fixed CO_2 . Therefore, photorespiration is a highly wasteful process. This happens only in case of C_3 plants. C_4 plants have overcome the problem of photorespiration by evolving O_2 or performing light reaction in mesophyll cells and Rubisco mediated CO_2 -fixation by Calvin Cycle in the interior of leaves (bundle sheath cells) where both temperature and oxygen are lower. They have further ensured high CO_2 supply to cells performing Calvin cycle. (ii) It helps in dissipation of energy where stomata get closed during daytime because of water stress. (iii) Photorespiration protects the plant from photooxidative damage by dissipating excess of excitation energy (Kazaki and Takeba, 1996).

Differences between Carboxylation and Oxygenation

Carboxylation (PCR Cycle)	Oxygenation (PCO Cycle)
1. It brings about assimilation of CO_2. 2. Oxygen is released. 3. It is an anabolic process and performs photosynthesis. 4. It stores energy. 5. Carboxylation occurs inside chloroplasts. 6. Only one type of organelles are involved. They are chloroplasts. 7. It is favoured by high CO_2 concentration. 8. Optimum temperature is lower, 18–35°C.	1. It releases CO_2. 2. Oxygen is consumed. 3. It is a catabolic process and performs photorespiration. 4. It wastes energy. 5. Initial oxygenation occurs inside chloroplasts. Second oxidation takes place in peroxisomes. 6. Three types of organelles are involved. They are chloroplasts, peroxisomes and mitochondria. 7. It is favoured by low CO_2 concentration. 8. Oxygenation occurs when temperature is higher, more than 35°C.

Photorespiration and Respiration. Photorespiration has no relation with normal respiration which occurs throughout day and night in all types of cells. However, in order to differentiate, normal respiration is often called **dark respiration** though the latter occurs in the illuminated cells as well.

Differences between Photorespiration and Normal (Dark) Respiration

Photorespiration	Dark (Normal) Respiration
1. It occurs only inside photosynthetic cells. 2. Photorespiration takes place only in the presence of light. 3. It is pronounced in C_3 plants and negligible in C_4 plants. 4. In photorespiration uptake of oxygen and evolution of carbon dioxide are light dependent. 5. Photorespiration increases with the availability of oxygen. 6. It occurs in the chloroplasts and may require the help of peroxisomes and mitochondria. 7. The substrate for photorespiration is RuBP and its breakdown product called glycolate.	1. It is found in all living cells. 2. This type of respiration continues both in dark and light. 3. It is pronounced in all organisms. 4. Exchange of gases is independent of light. 5. It is not influenced by change in oxygen concentration between 10–25%. Beyond it there is a decline in the rate of respiration. 6. It occurs in cytoplasm and mitochondria. 7. The substrate is commonly glucose though other food materials (fat, protein, organic acids) can also be used.

8. The substrate is formed at the time of utilization.
9. Toxic H_2O_2 may be formed during oxidation of the substrate.
10. End products are CO_2 and PGA.
11. Both the end products are reutilized in photosynthesis.
12. Photorespiration requires three cell organelles—chloroplasts, peroxisomes and mitochondria.
13. It is a wasteful method and does not produce energy.
14. It rises rapidly with the rise in temperature.
15. Photorespiration is not essential.

8. The substrate is already present in the cells.
9. Hydrogen peroxide is not produced.
10. End products are CO_2 and water.
11. The end products are not recycled in respiration.
12. It requires only mitochondria besides cytoplasm.
13. It produces energy for cellular maintenance, synthesis and work.
14. The rise with temperature is normal as for other biochemical reactions.
15. Normal or dark respiration is essential for survival of organisms.

C_4 -Dicarboxylic Acid Pathway (Hatch Slack Pathway, C_4 Pathway)

It was worked out by Hatch and Slack (1965, 1967). However, initial discovery was made by Kortschak *et al* (1965) who found that labelled carbon dioxide ($^{14}CO_2$) assimilated by Sugarcane leaves first appeared in a 4-carbon compound oxalo-acetic acid (OAA or oxaloacetate). Because of the initial discovery by Kortschak, this pathway of carbon assimilation is also called **HSK (Hatch Slack Kortschak) pathway**. Hatch and Slack found it a regular mode of CO_2 -fixation in a number of tropical plants, both monocots and dicots, e.g. [Maize, Sugarcane, Sorghum, *Panicum*, *Pennisetum* (Pearl Millet), *Atriplex*, *Amaranthus*, *Salsola*, etc.] These plants are called C_4 plants because of the first stable photosynthetic product being a 4-carbon compound. Other plants are C_3 plants. C_4 plants live in hot moist or arid and nonsaline or saline habitats. They have **Kranz anatomy** (Fig. 13.27). In Kranz anatomy, the

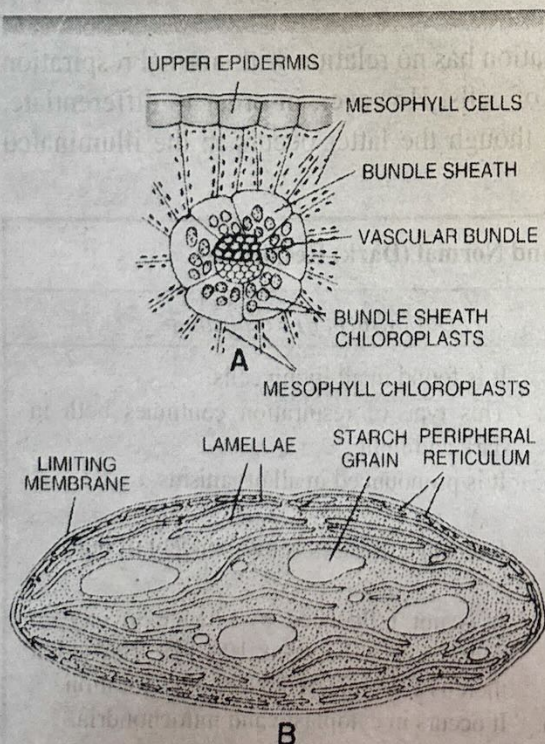


Fig. 13.27. A, part of C_4 leaf showing mesophyll and bundle sheath cells. B, structure of a typical bundle sheath chloroplast.

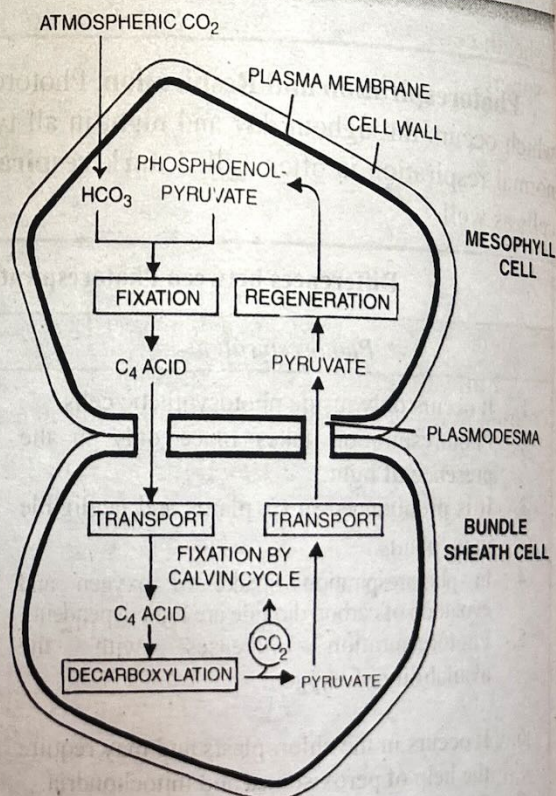
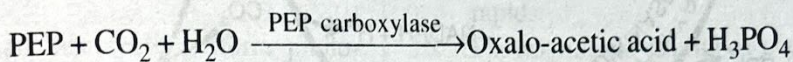


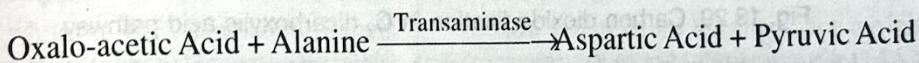
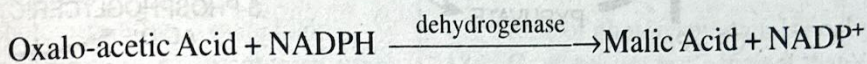
Fig. 13.28. Processes of C_4 photosynthesis in the two types of cells, mesophyll and bundle sheath cells.

mesophyll is undifferentiated and its cells occur in concentric layers around vascular bundles. Vascular bundles are surrounded by large sized **bundle sheath cells** which are arranged in a wreath-like manner (*kranz*—wreath) in one to several layers. The mesophyll and bundle sheath cells are connected by plasmodesmata or cytoplasmic bridges. The chloroplasts of the mesophyll cells are smaller. They have well developed grana and a peripheral reticulum but no starch. Mesophyll cells are specialised to perform light reaction, evolve O_2 and produce assimilatory power (ATP and NADPH). They also possess enzyme PEPCase for initial fixation of CO_2 . The chloroplasts of the bundle sheath cells are agranal. They possess a peripheral reticulum and starch grains. Thylakoids occur as stroma lamellae. ATP can be synthesised through cyclic photophosphorylation. However, photolysis and O_2 evolution are absent. Rather, bundle sheath cells are well protected from O_2 being released from mesophyll cells. Bundle sheath cells possess Rubisco.

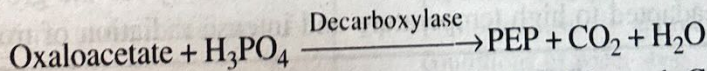
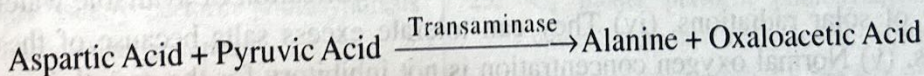
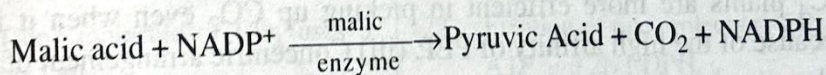
Initial Fixation. In C_4 plants, **initial fixation** of carbon dioxide occurs in mesophyll cells. The **primary acceptor** of CO_2 is **phosphoenol pyruvate** or PEP. It combines with carbon dioxide in the presence of PEP carboxylase or **PEPcase** to form oxalo-acetic acid or oxaloacetate.



Oxalo-acetic acid is reduced to malic acid or transaminated to form aspartic acid.

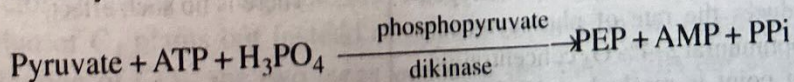


Transport. Malic acid or aspartic acid is translocated to bundle sheath cells through plasmodesmata. Inside the bundle sheath cells they are decarboxylated (and deaminated in case of aspartic acid) to form pyruvate and CO_2 . Since a number of mesophyll cells are feeding bundle sheath cells, the latter come to have a carbon dioxide concentrations several times that of atmosphere.



Final Fixation. CO_2 released in bundle sheath cells is fixed through Calvin cycle. RuBP of Calvin cycle is called **secondary or final acceptor** of CO_2 in C_4 plants.

Regeneration of PEP. Pyruvate and PEP formed in bundle sheath cells are sent back to mesophyll cells. Here, pyruvate is changed to phosphoenol pyruvate. Energy is required for this. The same is provided by ATP. The latter is changed into AMP (adenosine monophosphate).



Conversion of AMP to ATP requires double the energy than energisation of ADP to ATP. Therefore, actual requirement of energy is equal to two molecules of ATP. This energy is in addition to 3 ATP required for fixation of one molecule of CO_2 through Calvin cycle. Therefore, C_4 plants consume 5 ATP molecules per molecule of CO_2 fixed instead of 3 ATP molecules for C_3 plants. For the formation of a glucose molecule, C_4 plants require 30 ATP while C_3 plants utilize only 18 ATP.

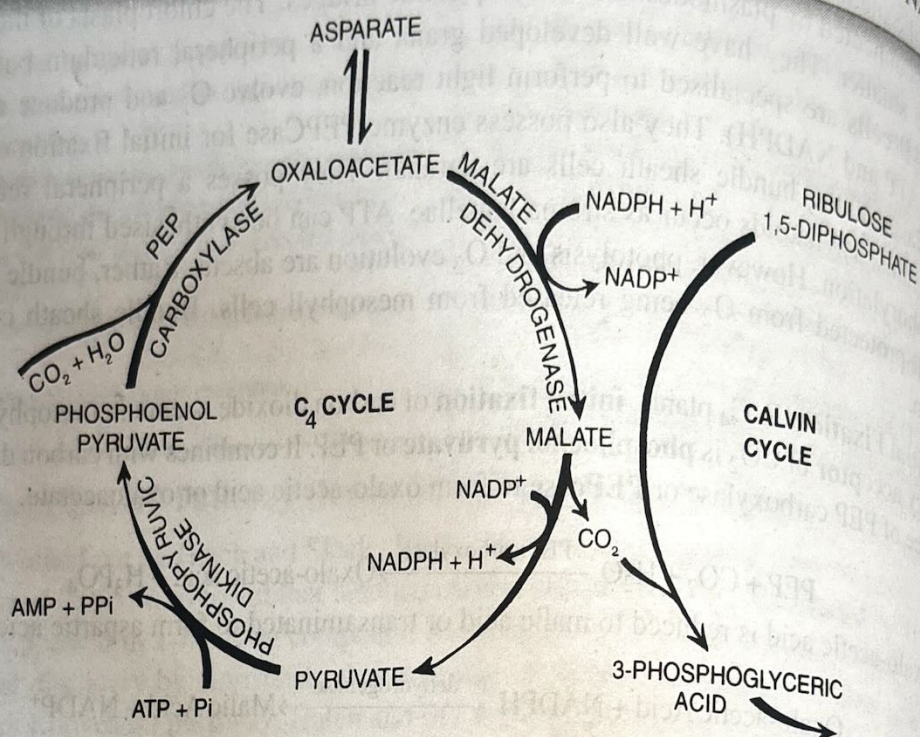
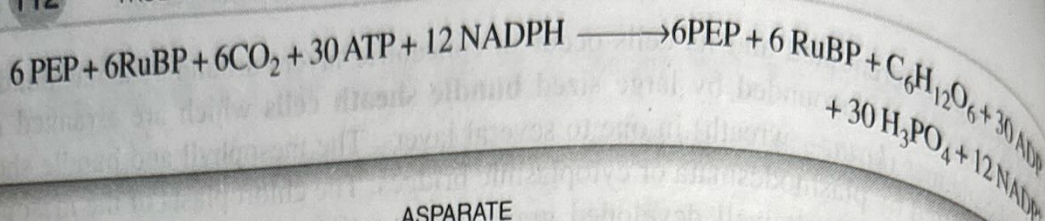


Fig. 13.29. Carbon dioxide fixation in C_4 dicarboxylic acid pathway.

Importance. (i) C_4 plants have a disadvantage. They consume more energy (2 more ATP molecules per molecule of CO_2 fixed). However, sufficient energy is available in the tropics where the plants grow. Further, C_4 plants have little photorespiration while in C_3 plants, more than half of photosynthetic carbon may be lost in photorespiration. C_4 pathway, is therefore, of adaptive advantage. (ii) C_4 plants are more efficient in picking up CO_2 even when it is found in low concentration because of the high affinity of PEP. (iii) Concentric arrangement of mesophyll cells produces a smaller area in relation to volume for better utilization of available water and reduce the intensity of solar radiations. (iv) They can tolerate excess salts because of the presence of organic acids. (v) Normal oxygen concentration is not inhibitory for the growth in contrast to C_3 plants. (vi) They are adapted to high temperature and intense radiation of tropics.

Differences between C_3 and C_4 Plants

C_3 Plants (Calvin Cycle)	C_4 Plants (Hatch Slack Cycle)
- Optimum temperature for photosynthesis is $10-25^\circ\text{C}$. - Atmospheric concentration of oxygen (21%) reduces the rate of photosynthesis which is optimum at 2-4% O_2 concentration. - Saturation point is reached at 10-70% of full sunlight. - Rate of net photosynthesis at optimum sunlight is $15-35 \text{ mg CO}_2/\text{dm}^2/\text{hr}$. - The leaves do not possess Kranz anatomy. - Chloroplasts do not have peripheral reticulum.	- Optimum temperature is $30-45^\circ\text{C}$. - There is no such effect. - Saturation point is not reached even at full sunlight. - Rate of net photosynthesis at full sunlight is $40-80 \text{ mg CO}_2/\text{dm}^2/\text{hr}$. - The leaves have Kranz anatomy. - Chloroplasts have peripheral reticulum.

7. Chloroplasts are of one type (monomorphic).
8. Bundle sheath cells usually do not contain chloroplasts.
9. In higher plants operating C_3 cycle, the chloroplasts are all granal.
10. Ribulose biphosphate is the first acceptor of CO_2 .
11. Phosphoglyceric acid is the first product.
12. The plants operate only Calvin cycle.
13. CO_2 compensation point is 25–100 ppm.
14. Mesophyll cells perform complete photosynthesis.
15. The rate of carbon assimilation is slow.
16. The plants are unable to perform photosynthesis at very low CO_2 concentration (say 10–50 ppm).
17. Rate of photorespiration is quite high.
18. At low temperature, C_3 plants are more efficient while at high temperature their photosynthetic activity is comparatively reduced.
19. The cycle operates in all plants.
20. The plants are adapted to all types of climates.
21. C_3 plants usually cannot tolerate saline conditions.
22. Fixation of one molecule of CO_2 uses 3 ATP and 2NADPH.
23. C_3 plants usually perform photosynthesis only when stomata are open.
24. Photosynthesis stops under conditions of water stress.

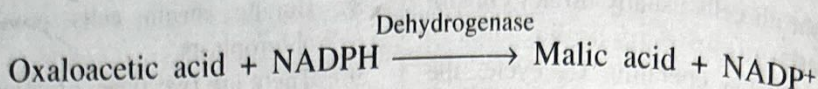
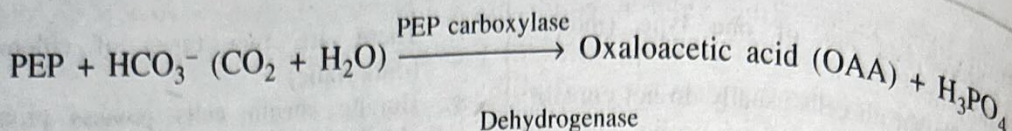
7. There are two types of chloroplasts (dimorphic).
8. Bundle sheath cells possess prominent chloroplasts.
9. There are two types of chloroplasts, granal in mesophyll cells and agranal in bundle sheath cells.
10. Phosphoenol pyruvate is the first acceptor of CO_2 , while ribulose biphosphate is the second acceptor.
11. Oxalo-acetic acid is the first product.
12. Plants operate a dicarboxylic acid cycle in addition to Calvin cycle.
13. CO_2 compensation point is 0–10 ppm.
14. Mesophyll cells perform only initial fixation.
15. The rate of carbon assimilation is quite rapid.
16. Photosynthesis continues even at very low CO_2 concentration of 10–50 ppm.
17. Rate of photorespiration is negligible.
18. C_4 plants are less efficient than C_3 plants at low temperature but they have a higher net assimilation at high temperature.
19. The cycle is found only in some plants.
20. The plants are adapted to tropical climate.
21. C_4 plants can tolerate halophytic conditions.
22. Fixation of one molecule of CO_2 requires 5 ATP and 2NADPH.
23. C_4 plants perform photosynthesis even when stomata are closed (from CO_2 produced in respiration). → PEP TAKES FROM HCO_3^- UNLIKE RUBISCO
24. Photosynthesis is appreciable even under conditions of water stress.

Crassulacean Acid Metabolism (Fig. 13.30)

It is a mechanism of photosynthesis involving double fixation of CO_2 which occurs in succulents belonging to crassulaceae, cacti, euphorbias and some other plants of dry habitats where the stomata remain closed during the daytime and open only at night. The process of photosynthesis is similar to that of C_4 plants but instead of spatial separation of initial PEPcase fixation and final Rubisco fixation of CO_2 , the two steps occur in the same cells but at different times, night and day, e.g., *Sedum*, *Kalanchoe*, *Opuntia*, Pineapple, Agave, Vanilla. Ice plant (*Mesembryanthemum crystallinum*) is a facultative CAM plant. It performs C_3 photosynthesis under normal conditions and CAM metabolism under stress conditions (of water, heat and salt).

Phase I. Stomata are open at night. CO_2 is absorbed from outside. With the help of PEP carboxylase, it is immediately fixed. The acceptor is phosphoenol pyruvate or PEP.

TEMPORAL SEPARATION



Malic acid is end product of dark fixation of CO_2 . It is stored inside cell vacuoles.

Phase II. During daytime, stomata are closed. However, light is available for photosynthesis. Malic acid moves out of the cell vacuoles. It is decarboxylated with the help of malic enzyme. Pyruvate is produced. It is metabolised.

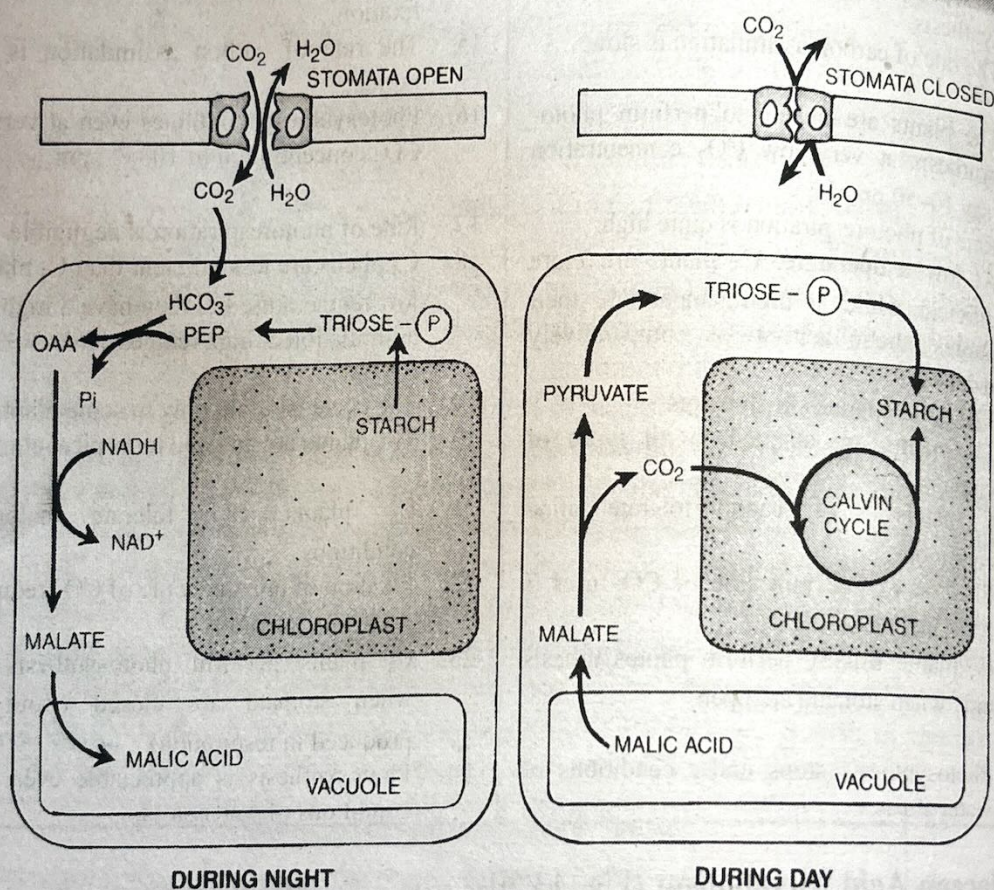
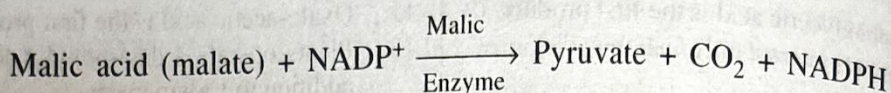


Fig. 13.30. Mechanism of Crassulacean acid metabolism showing CO_2 uptake from outside during night and its utilisation in photosynthesis during daytime.

The released CO_2 is again fixed through Calvin cycle with the help of RuBP and Rubisco. Crassulacean plants have developed a unique mechanism to perform photosynthesis without much loss of water which occurs in other modes of photosynthesis. They perform **acidification** or dark fixation of CO_2 during night (Phase I) and **deacidification** (Phase II) during day to release CO_2 for actual photosynthesis.

Cardinal Points (Fig. 13.31). Sachs (1860) found that a factor influencing a physiological process has three principal values called cardinal points— **minimum**, **optimum** and **maximum**. The minimum of a factor is that value below which the physiological process cannot continue.

Maximum of a factor is that value beyond which the process comes to stop. Optimum value of the factor is that point where the physiological process can continue indefinitely at its highest velocity.

Principle or Law of Limiting Factors

Optimum value of a factor is never constant. It depends upon the magnitude of other factors. We may continue to increase the magnitude of one or more factors without influencing the rate of reaction. In such cases it is found that a factor called limiting factor is holding the balance. A limiting factor is defined as a factor which is deficient to such an extent that increase in its magnitude directly increases the rate of the process. Blackman in 1905. He formulated the principle of limiting factors, which states that when a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor. In other words the rate of a physiological process is limited at a given time by one and only one factor which is deficient.

Blackman (1905) studied the effect of CO_2 concentration, light intensity and temperature on rate of photosynthesis. All other factors were maintained in optimum concentration. Initially the photosynthetic material was kept at 20°C in an environment having 0.01% CO_2 . When no light was provided to photosynthetic material, it did not perform photosynthesis. Instead, it evolved CO_2 and absorbed O_2 from its environment. Blackman provided light of low intensity (say 150 foot candles*) and found photosynthesis to occur. When light intensity was increased (say 800 foot candles), the rate of photosynthesis increased initially but soon it levelled off. The rate of photosynthesis could be further enhanced only on the increase in availability of CO_2 . Thus, initially light intensity was limiting the rate of photosynthesis. When the same was available in sufficiency, CO_2 became limiting (Fig. 13.32). When both were provided in sufficient quantity, the rate of photosynthesis rose initially but again reached a peak. It could not be increased further. At this time, it was found that increase in temperature could raise the rate of photosynthesis upto

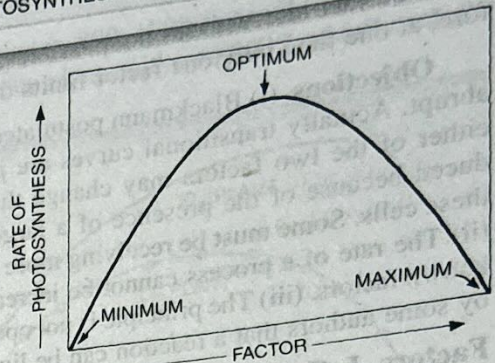


Fig. 13.31. The three cardinal points.

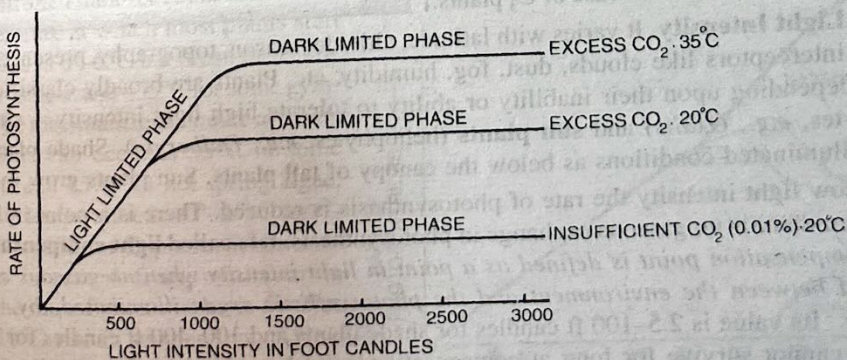


Fig. 13.32. Blackman's law of limiting factors.

* 19 foot candles = 1000 watt/m = 1 W/m; 1 ft candle = 10.76 lux.

35°C. Further increase was not possible. At this stage, some other factor became limiting. Therefore, at one time only one factor limits the rate of a physiological process.

Objections. (i) Blackmann postulated that change over from one limiting factor to another is abrupt. Actually transitional curves are present in such regions. During transition, a change in either of the two factors may change the rate of the process. The transitional curves are produced because of the presence of a large number of green cells and numerous chloroplasts in these cells. Some must be receiving more light than others while a few must be getting more CO_2 . (ii) The rate of a process cannot be increased indefinitely by increasing the availability of all the known factors. (iii) The principle is not operative for toxic chemicals or inhibitors. (iv) It is believed by some authors that a reaction can be limited simultaneously by two or more factors.

Factors Influencing Photosynthesis

External Factors (Environmental Factors)

1. **Carbon Dioxide.** CO_2 concentration of the atmosphere is 0.036% or 360 ppm ($360 \mu\text{L l}^{-1}$). It is a limiting factor for C_3 as the available CO_2 concentration is lower than the optimum for photosynthesis. Increase in its concentration upto 0.05% increases the rate of photosynthesis in most C_3 plants (Fig. 13.33). A decline is observed beyond 0.1%. When CO_2 concentration is reduced, there comes a point at which illuminated plant parts stop absorbing carbon dioxide from their environment. It is known as CO_2 **compensation point** or **threshold value**. At this value CO_2 fixed in photosynthesis is equal to CO_2 evolved in respiration and photorespiration. The value is 25–100 ppm in C_3 plants and 0–10 ppm in C_4 plants. The reason for low compensation value for C_4 plants is the greater efficiency of CO_2 -fixation through PEP-carboxylase. The optimum CO_2 concentration for C_4 plants is $360 \mu\text{L l}^{-1}$ or 360 ppm. However, the atmospheric concentration of CO_2 is increasing. It has already reached 380 ppm. If the trend continues, CO_2 concentration may reach 600 ppm after a few decades. It will be harmful to C_4 plants while productivity is going to increase in case of C_3 plants.

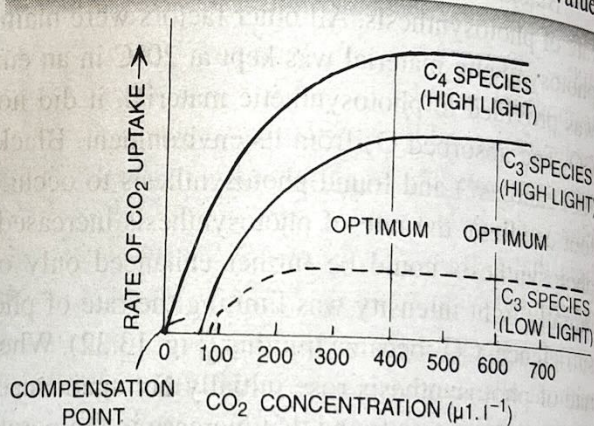


Fig. 13.33. Effects of CO_2 concentration on photosynthesis of C_3 and C_4 plants.

2. **Light Intensity.** It varies with latitude, altitude, season, topography, presence or absence of light interceptors like clouds, dust, fog, humidity, etc. Plants are broadly classified into two groups depending upon their inability or ability to tolerate high light intensity—**shade plants** (sciophytes, e.g., *Oxalis*) and **sun plants** (heliophytes, e.g., *Dalbergia*). Shade plants grow in poorly illuminated conditions as below the canopy of tall plants. Sun plants grow in the open.

At low light intensity the rate of photosynthesis is reduced. There is a point in light intensity where there is no gaseous exchange in photosynthesis. It is called **light compensation point**. *Light compensation point is defined as a point in light intensity when no gaseous exchange is observed between the environment and the photosynthetic organ illuminated by that light intensity.* Its value is 2.5–100 ft candles for shade plants and 100–400 ft candles for sun plants. A plant cannot survive for long at compensation point because there is a net loss of organic matter due to respiration of nongreen organs and respiration in dark.

As the light intensity ($\mu\text{mol. m}^{-2}\text{s}^{-1}$) increases, the rate of photosynthesis ($\mu\text{mol. CO}_2\text{ m}^{-2}\text{s}^{-1}$) also increases. The light intensity at which a plant can achieve maximum amount of

photosynthesis is called **light saturation point**. Its value is 800–1000 ft candles (10% of full sunlight) in shade plants, 50–70% of full sunlight in C_3 sun plants and upto 200% of full sunlight in C_4 sun plants, (e.g., Sugarcane, Fig. 13.34).

Beyond saturation point (it is seldom realised in nature in C_4 sun plants) the rate of photosynthesis begins to decline. The phenomenon is called **solarisation**. It is due to two reasons: (i) **Photo-inhibition** due to reduction in hydration and closure of stomata (ii) **Photo-oxidation** or oxidation of photosynthetic pigments, intermediates and enzymes. Carotenoids are important in protecting against photooxidation by (a) Acting as antioxidants (absorbing activated oxygen and releasing the same as molecular oxygen) and preventing oxidation of chlorophyll and other molecules. (b) Absorbing excess light energy and diverting the same away from chlorophyll.

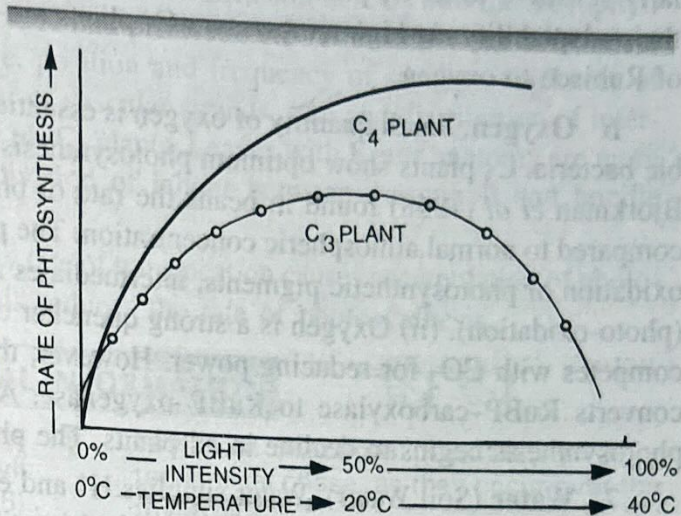


Fig. 13.34. Comparison between the rate of photosynthesis in C_3 and C_4 plants at different temperatures and light intensities.

3. **Quality of Light.** The range of **photosynthetically active radiation** (PAR) lies between 400 – 700 nm. Maximum photosynthesis occurs in blue-violet and red regions of the light spectrum where most of the absorption is carried out by chlorophylls. Red light favours carbohydrate accumulation while blue light stimulates protein synthesis. Minimum photosynthesis occurs in the green wavelengths. Plants growing under the canopy of other trees receive very little red and blue-violet light because of its absorption by leaves of the canopy. They receive more of green light that is transmitted through the tree leaves. As a result the rate of photosynthesis of herbs, shrubs and other undergrowths in a forest is comparatively low. Ultra-violet rays are harmful.

4. **Duration of Light.** Continuous photosynthesis can occur in continuous illumination without any harm to the plant (Bohning, 1949) though the rate of photosynthesis may slightly decline after six days.

5. **Temperature.** It does not influence light reactions of photosynthesis but affects the enzyme-controlled dark reactions. The minimum temperature at which most plants start photosynthesis is 0°C – 5°C but it can be as low as -20°C for lichens and -35°C for some gymnosperms. The maximum temperature at which photosynthesis can occur is 55°C in some desert plants and 75°C for hot spring algae. The optimum temperature is 10°C – 25°C for C_3 plants and 30°C – 45°C for C_4 plants. When temperature is increased from minimum to optimum, the rate of photosynthesis doubles for every 10°C rise in temperature. Above the optimum temperature, the rate of photosynthesis shows an initial increase for short duration but later declines. This decline with time is called **time factor** (Fig. 13.35).

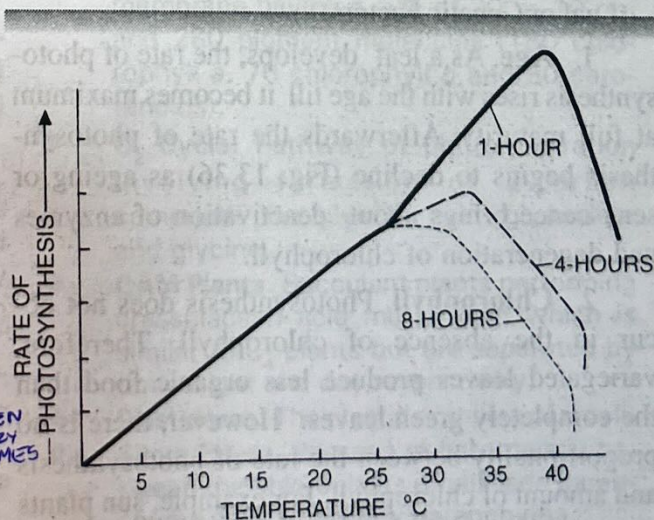


Fig. 13.35. Effect of temperature and time factor on photosynthesis.

At low temperature enzymes become inactive. C_4 plants show little photosynthesis even at not so low temperature ($2-10^\circ\text{C}$) because their enzyme pyruvate phosphate dikinase is particularly sensitive to it. C_3 plants show different responses to lower temperatures depending upon their adaptability. At high temperature C_3 plants are more affected because of increased affinity of Rubisco to oxygen.

6. **Oxygen.** Small quantity of oxygen is essential for photosynthesis except in some anaerobic bacteria. C_3 plants show optimum photosynthesis at low oxygen concentration. For example, Bjorkman *et al* (1968) found in beans the rate of photosynthesis at 2.5% oxygen was twice as compared to normal atmospheric concentration. The possible reasons are (i) Oxygen takes part in oxidation of photosynthetic pigments, intermediates and enzymes in the presence of strong light (photo-oxidation). (ii) Oxygen is a strong quencher of excited state of chlorophyll. (iii) Oxygen competes with CO_2 for reducing power. However, this effect is not known in C_4 plants. (iv) It converts RuBP-carboxylase to RuBP-oxygenase. At a very high oxygen content the rate of photosynthesis begins to decline in all plants. The phenomenon is called **Warburg effect**.

7. **Water** (Soil Water). Water supplies H^+ and electrons for carbon dioxide fixation. However, less than 1% of the total water absorbed is utilized in photosynthesis. The rest is lost in transpiration. Even a slight increase in transpiration reduces the leaf hydration that cuts down photosynthesis by causing stomatal closure and hence decreased CO_2 absorption, loss of leaf turgidity, reduced absorption of solar radiations and decreased enzymatic activity. There is reduction in leaf water potential. It reduces leaf growth and hence photosynthetic area. Thus photosynthesis is more sensitive to dehydration than any other metabolic process (Rabinowitch, 1945).

8. **Air Pollutants.** Dust and smoke particles present in the atmosphere reduce photosynthesis by reducing light penetration and forming a layer over the plants. Sulphur dioxide, nitrogen oxides, hydrogen fluorides and other air pollutants also decrease photosynthesis.

9. **Minerals** (Nutrient Supply). Nitrogen is component of chlorophyll, enzymes, electrons transport chains and various structures of photosynthetic cells. Magnesium is a component of chlorophyll. Fe, Cu and Mn are required for synthesis of chlorophyll. Mn and Cl are linked to photolysis of water. P as phosphate is essential for ATP synthesis. Enzyme activators of photosynthesis include potassium and sulphur. Lower availability of any of these minerals reduces rate of photosynthesis.

Internal or Plant Factors

(Leaf or Genetic Factors)

1. **Age.** As a leaf develops, the rate of photosynthesis rises with the age till it becomes maximum at full maturity. Afterwards the rate of photosynthesis begins to decline (Fig. 13.36) as ageing or senescence brings about deactivation of enzymes and degeneration of chlorophyll.

2. **Chlorophyll.** Photosynthesis does not occur in the absence of chlorophyll. Therefore, variegated leaves produce less organic food than the completely green leaves. However, there is no proportionality between the rate of photosynthesis and amount of chlorophyll. For example, sun plants contain less chlorophyll as compared to shade plants but the rate of photosynthesis in bright light is much higher in sun plants than in shade plants.

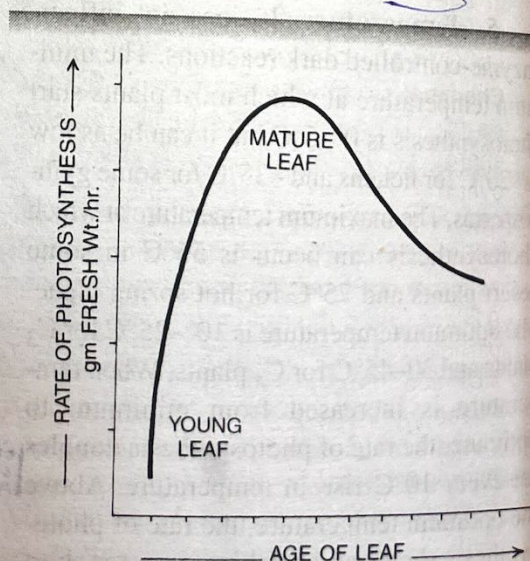


Fig. 13.36. Relation between age of leaf and rate of photosynthesis.

3. **Hormones.** Cytokinins and gibberellins increase the rate of photosynthesis but abscisic acid reduces the same.

4. **Leaf Anatomy.** Leaf anatomy influences the rate of diffusion of CO_2 into the mesophyll cells, availability of light, rate of translocation of end products, etc. The important anatomical structures influencing them are size, structure, position and frequency of stomata, thickness of epidermis and cuticle, distribution and number of vascular strands, size and distribution of inter-cellular spaces, etc. Kranz anatomy is specific for C_4 plants. Leaves with Kranz anatomy are more efficient in photosynthesis because of the division of labour between mesophyll and bundle sheath cells.

5. **Accumulation of End Products.** Slow rate of translocation causes accumulation of photosynthetic end products during afternoon. This reduces the rate of photosynthesis.



ADDITIONAL INFORMATION



- **G.G. Stokes** (1864). First to separate pure fractions of chlorophyll *a* and chlorophyll *b*.
- **James Clark Maxwell** (1860, 1865) proposed electromagnetic wave theory.
- **Willstatter and Stoll** (1912). They studied the structure of photosynthetic pigments.
- **DCMU**. Dichlorophenyl dimethyl urea — a herbicide.
- **Thylakoids** (Menke, 1961). Structural (and functional) units of chloroplasts.
- **Size of Chlorophyll**. Porphyrin head is $15\text{\AA} \times 15\text{\AA}$ while phytol tail is $20\text{\AA}$ long.
- **Quantum Yield**. Number of oxygen molecules released per photon or quantum of light is known as quantum yield. Its value is $1/8$ – $1/10$ (10–12.5%). In other words evolution of one molecule of oxygen or consumption/fixation of one molecule of CO_2 requires 8–10 quanta. The number of light quanta used for producing one molecule of O_2 or fixation of one molecule of CO_2 is called **quantum requirement**.
- **Chemical Coupling Hypothesis**. Synthesis of ATP is connected to liberation of energy while electrons pass down hill over the electron transport chain.
- **Chemi-Osmotic Hypothesis** (Mitchell, 1961). There is accumulation of protons which pass through membrane based ATPase (or coupling factor, elementary particle) catalysing synthesis of ATP from ADP and inorganic phosphate.
- **Phaeophytin**. It is chlorophyll in which central magnesium is replaced by two hydrogen atoms.
- **Cytochrome**. Kellin (1925) coined the term.
- **Oxidation of Water**. In photosynthesis water is oxidised to produce hydrogen for reducing CO_2 . O_2 is evolved in the process.
- **Thylakoid Reactions**. Reactions of photochemical phase, as they occur over the thylakoids.
- **Stroma Reactions**. Reactions of biosynthetic phase, as they occur in stroma.
- **Wavelength and Frequency**. Light of shorter wavelength has higher frequency while that of longer wavelength has lower frequency.
- **P_{700}** . Discovered by Bessel Kok.
- **P_{682} or P_{680}** . Discovered by Witt *et al.*
- **Diatoms**. Reinfelder *et al* (2000) has found that diatoms perform C_4 photosynthesis.
- **P-protein**. It is synthesised in companion cells (absent in gymnosperms). In sieve tube cells it has a role as defence against phloem feeding insects and sealing of damaged sieve tubes by plugging sieve plates.
- **Quantasome**. Believed by Park and Biggins (1963) to be separable piece of thylakoid membrane having a size of 20×10 nm and 280 pigment molecules (160 chlorophyll *a*, 70 chlorophyll *b* and 50 carotenoids).
- **C_2 Cycle**. Pathway of photorespiration involving formation of 2-carbon compounds like glycolic acid, glyoxylate and glycine.
- **CAM Plants**. Succulent plants performing crassulacean acid metabolism which is similar to C_4 plants but are separated by time, night and day respectively.
- **Plastidome**. The plastid complex of a cell.
- **Sieve Effect**. Passage of light rays in between the chloroplasts of palisade parenchyma cells to spongy parenchyma.
- **Utilisation of Light Energy**. Only 4–15% of absorbed light energy is used in photosynthesis.

NCERT TEXTBOOK QUESTIONS WITH ANSWERS

1. By looking at a plant externally can you tell whether a plant is C_3 or C_4 . Why and how ?
 ✓ External morphology does not necessarily differentiate C_3 and C_4 plants. C_4 leaves are isobilateral but all isobilateral leaves are not C_4 in nature. Leaves of C_3 plants are generally dorsiventral. C_4 plants are tropical plants while C_3 plants occur in all zones of the earth. Many C_4 plants are found in hot, arid and saline habitats.

2. By looking at which internal structure of a plant can you tell whether a plant is C_3 or C_4 ? Explain.
 ✓ Internal structure of **leaf** can reveal whether it belongs to C_3 or C_4 plant. In C_4 plants a leaf has **Kranz anatomy** where the undifferentiated mesophyll occurs in concentric layers around vascular bundles. Each vascular bundle is surrounded by a bundle sheath of large sized green cells. The chloroplasts of the bundle sheath cells are devoid of grana. They, however, contain starch grains. The chloroplasts of the mesophyll cells are granal and starch grains do not occur in them. In C_3 plants, bundle sheath cells are neither very large, nor they contain chloroplasts. Mesophyll is often differentiated into palisade and spongy parenchyma. All chloroplasts are granal and possess starch grains.

3. Even though a very few cells in a C_4 plant carry out biosynthetic Calvin pathway, yet they are highly productive. Can you discuss why ?

✓ In C_4 plants, biosynthetic Calvin cycle occurs only in bundle sheaths. Despite few number of cells performing Calvin cycle, C_4 plants have a high rate of photosynthesis due to (i) Rapid supply of CO_2 from all mesophyll cells taking part in initial carbon dioxide fixation. (ii) Absence of photorespiration due to absence of photolysis in bundle sheath cells. (iii) Rapid withdrawal of photosynthates from the bundle sheath cells as they lie over the vascular bundles. (iv) Photosynthesis continues even when stomata are closed due to fixation of CO_2 released through respiration.

4. RuBisCo is an enzyme that acts both as a carboxylase and oxygenase. Why do you think RuBisCo carries out more carboxylation in C_4 plants ?

✓ RuBisCO functions as oxygenase only when there is higher concentration of oxygen and lower concentration of CO_2 . Both the conditions do not occur in RuBisCO containing bundle sheath cells of C_4 plants. They do not have photolytic evolution of oxygen. They receive a regular supply of CO_2 even when the stomata are closed. Therefore, RuBisCO of C_4 plants functions only as carboxylase.

5. Suppose there were plants that had a high concentration of chlorophyll *b* but lacked chlorophyll *a*. Would it carry out photosynthesis ? Then, why do plants have chlorophyll *b* and other accessory pigments ?

✓ Chlorophyll *a* is essential for photosynthesis because only its special molecules function as reaction centres (P_{700} , P_{680}). Reaction centres convert light energy into chemical or electron energy. Photosynthesis is, therefore, absent in plants lacking chlorophyll *a*.

The importance of chlorophyll *b* and other accessory pigments is (i) Absorption of light rays of different wavelengths and transfer of this energy to reaction centre through resonance. (ii) Protection of chlorophyll *a* reaction centre from nascent oxygen and other destructive radicals.

6. Why is the colour of a leaf kept in the dark frequently yellow or pale green ? Which pigment do you think is more stable ?

✓ A leaf kept in dark for long becomes yellow or pale green because of the disintegration of chlorophylls. Carotenoids, which provide yellow colour to the leaf are more stable.

7. Look at leaves of the same plant on the shady side and compare it with the leaves on the sunny side or compare the potted plants kept in the sunlight with those in the shade. Which of them has leaves that are darker green ? Why ?

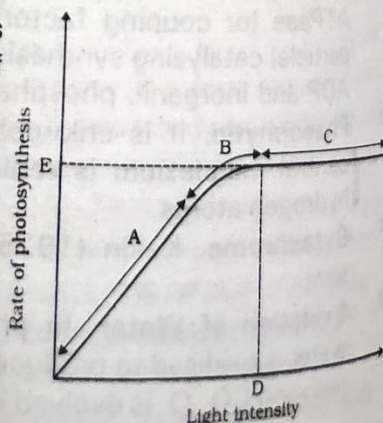
✓ Shaded leaves are dark green while the sun exposed leaves are tinged light green due to two reasons. (i) Photooxidation of chlorophyll in bright light and its nonoxidation in shaded region. (ii) Orientation of chloroplasts in vertical files along the walls in strong light and their irregular arrangement in shade.

8. Figure shows the effect of light on the rate of photosynthesis. Based on the graph, answer the following questions :

(a) At which point/s (A, B or C) in the curve is a light limiting factor.

(b) What could be the limiting factor/s in the region A.

(c) What do C and D represent on the curve.



- ✓ (a) Light is limiting in A and 50% of B. (b) Light. (c) C – Factor other than light (like CO_2) is limiting.
 D – Light saturation point under existing conditions of other factors.
9. Give comparison between the following : (a) C_3 and C_4 pathways. (b) Cyclic and noncyclic photophosphorylation. (c) Anatomy of leaf in C_3 and C_4 plants.
- ✓ (a) **Comparison between C_3 and C_4 pathway.** Points 1–4, 10–18 and 22–24 of table given in the text. (b) **Differences between Cyclic and Noncyclic Photophosphorylation,** Refer to the text. (c) **Differences in the Anatomy of C_3 and C_4 Plants.** Points 5 – 9 in the table given in the text.

TEST QUESTIONS

One Mark Questions (With Answers)

- What amount of total Solar energy is falling on the earth per year ?
✓ 5×10^{20} Kcal./yr.
- Which form of carbon is absorbed by the hydrophytes through their general body surface ?
✓ Bicarbonates.
- Which chlorophyll is termed as universal photosynthetic pigment ?
✓ Chlorophyll *a*.
- Name the photosynthetic pigment which can be converted to Vitamin A.
✓ β -carotene.
- What is the site of formation of glyoxylate from glycolate ?
✓ Peroxisomes of mesophyll cells.
- Does photorespiration release energy ?
✓ No, rather it consumes energy.
- Name the central element present in chlorophyll.
✓ Magnesium.
- Are the enzymes that catalyse the dark reaction of carbon fixation located inside the thylakoids or outside the thylakoids ?
✓ Outside thylakoids, in the stroma.
- Mention one difference in the structure of chlorophyll *a* and chlorophyll *b*.
✓ Chlorophyll *a* has methyl ($-\text{CH}_3$) group where as chlorophyll *b* has aldehyde ($-\text{CHO}$) at carbon 3.
- Name two photosynthetic pigments belonging to carotenoids.
✓ Carotene and Xanthophyll.

One Mark Questions (Without Answers)

- Explain the terms : (a) Photosynthesis (b) Hill Reaction (c) Fluorescence (d) Photocentre (e) RuBisCo (f) Accessory Pigments.
- Name (a) A hill oxidant that is blue in oxidised state and colourless in reduced state (b) Photosynthetic pigments of bacteria (c) Primary photosynthetic pigment (d) Contribution of Hatch and Slack (e) Types of photophosphorylation.
- Expand the terms : (a) DCPIP (b) PGA (c) RuBP (d) PEP (e) OAA (f) NADP^+ (g) NAD (h) P_{700} (i) P_{680} (j) PS I (k) PS II.
- Define (a) Photon (b) Quantum (c) C_3 plant (d) C_4 plant (e) Light Reaction (f) Photorespiration (g) Limiting Factor.
- A plant was supplied with labelled carbon dioxide (CO_2). The heavier oxygen O_2^{18} will appear in (a) glucose molecule (b) water molecule (c) oxygen molecule (d) adenosine triphosphate.
- A destarched leaf and a potted plant is coated with vaseline on both sides and placed in sunlight. After a few hours it is tested for the presence of starch. Will the test be positive or negative ?
- Differentiate between absorption spectrum and action spectrum.
- Why are plants that consume more than usual 18 ATP to produce one molecule of glucose favoured in tropical regions ?
- Name the first stable product of C_4 cycle ?
- State Blackmann's Law of limiting factors.
- Name the reaction centres of PS I and PS II.
- How many molecules of ATP are required for synthesis of one molecule of glucose in C_3 and C_4 pathways ?

Two Mark Questions (With Sample Answers)

23. What is light? What part of light is most suitable for photosynthesis?
✓ Light is the visible part of electromagnetic radiations. Blue and red regions of the light spectrum are the most effective in photosynthesis.
24. All life on earth is 'bottled sunshine'. Justify.
✓ Photosynthesis converts radiant or solar energy into chemical energy. The same gets stored in the organic food as bonds between different atoms. Photosynthetic products provide energy to all organisms to carry out their life activities (all life is bottled sunshine).
25. Write the symbols for photosystem one and photosystem two. Which one of these can carry on photophosphorylation independently?
✓ PS I, PS II; PS I can carry cyclic photophosphorylation independently.
26. Why is photorespiration also called C_2 cycle?
✓ Since it involves synthesis of two carbon compounds like glycolic acid, glyoxylate and glycine.
27. Name the hormones which increase and decrease the rate of photosynthesis?
✓ Cytokinins and Gibberellins Increase, ABA decreases.

Two Mark Questions (Without Answers)

28. Explain why photosynthesis is considered the most important process in biosphere?
29. Specify how a pentose phosphate is a CO_2 acceptor in the dark reaction of photosynthesis.
30. Specify how C_4 photosynthetic pathway increases CO_2 concentration in bundle sheath cells of sugarcane.
31. What is RuBisCo? Explain its role in C_3 and C_4 photosynthesis.
32. Give the importance of C_4 plants.
33. Suggest an experiment to prove that organic food is translocated through phloem.

Three Mark Questions (Short Answer Type)

34. Distinguish between
 - (a) Respiration and photorespiration.
 - (b) Cyclic photophosphorylation and non-cyclic photophosphorylation.
35. (a) What is the difference between chlorophyll *a* and chlorophyll *b*?
(b) Specify how a pentose phosphate is a CO_2 -acceptor in the dark reaction of photosynthesis.
36. What is the advantage of having more than one pigment molecule in a photocentre.
37. What are the steps that are common to C_3 and C_4 photosynthesis?
38. Why are plants that consume more than usual 18 ATP to produce 1 molecule of glucose favoured in tropical regions?
39. A plant is believed to be releasing oxygen during night time. Do you believe in the truthfulness of this statement? Justify your answer by giving the reason.
40. A suspension of isolated chloroplasts was illuminated in the absence of carbon dioxide and DCPIP. Answer the following: (i) Will the colour suspension appear blue? (ii) Will the photolysis of water occur? (iii) Will the oxygen be released?
41. Differentiate between absorption spectrum and action spectrum.
42. What is photorespiration? How are photorespiratory losses overcome by C_4 plants?
43. Expand RuBP? Where is it found in a C_3 plant? What is its role in biosynthesis process?
44. RuBisCo carries on a dual role as a carboxylase as well as oxygenase. Explain both the reactions. Under what conditions does this shift from one role to the other take place?
45. Write a brief account on the chloroplast pigment?
46. How is C_4 pathway more energy expensive than C_3 cycle?
47. Give three advantages of C_4 cycle over C_3 cycle.
48. What is Kranz anatomy? Write a note on photosynthesis of those plants in which this anatomy is found.
49. Name any two C_4 plants. Give anatomical characters of these plants.
50. (a) What are photosystems? Explain in brief.
(b) Mention the advantages of having more than one pigment molecule in photocentre.
51. Describe the mechanism of Hatch and Slack pathway in C_4 plants.
52. Give an account of carboxylation (CO_2 fixation) stage in Calvin cycle in photosynthesis.
53. Distinguish between photosynthesis and respiration.

54. Describe cytoplasmic streaming hypothesis.

Five Mark Questions (Long Answer Type)

55. What is the law of limiting factors ? Explain it with an illustration.
56. Discuss the effect of various environmental and plant factors on the process of photosynthesis.
57. Why does chlorophyll appear green in transmitted light and red in reflected light ? Explain the significance of these phenomena in terms of photosynthesis.
58. Describe the light-dependent steps of photosynthesis. How are they linked to the dark reaction?
59. Calvin cycle consists of three phases. What are they ? Explain the significance of each of them.
60. Define carbon fixation in plants. What was the contribution of Melvin Calvin. Describe the three phases of Calvin cycle.
61. How are ATP, NADPH and oxygen produced in noncyclic photophosphorylation.
62. Describe in short the various steps that occur during the photochemical phase of photosynthesis.
63. (i) How many turns of dark reaction yield one mole of glucose ? (ii) Explain main chemical steps that take place in the dark reaction or Calvin cycle of photosynthesis.
64. Describe C_3 pathway of photosynthesis. How does it differ from C_4 pathway ?
65. Name any two C_4 plants. Describe how carbon dioxide is fixed in such plants.
66. What is photorespiration ? Explain the mechanism and its significance.
67. What is photorespiration ? How are photorespiratory losses overcome by C_4 plants ?
68. Give an account of factors which affect photosynthesis.
69. What is disadvantage of photorespiration ? Explain how the photorespiratory losses are overcome in plants like sugarcane.
70. Where does non-cyclic photophosphorylation take place ? Describe the process. Why is process referred to as non-cyclic ?
71. Explain the process of oxidation reduction occurring in photosynthesis to generate ATP and NADPH.
72. Explain photo-chemical phase of photosynthesis. Where does it take place ?
73. Describe the cyclic photophosphorylation. What is the purpose of proton gradient during the process in thylakoids ?
74. Give an account of external factors affecting photosynthesis.
75. Sugarcane has developed a mechanism to compensate photorespiratory losses. Name the mechanism and describe it stepwise.
76. What is meant by photophosphorylation ? Discuss the cyclic photophosphorylation.
77. Describe various steps of light reaction.
78. Where does cyclic photophosphorylation take place in the leaves ? Why is this process called cyclic? Describe cyclic photophosphorylation.
79. In an experiment radioactive $^{14}CO_2$ was supplied to green plants. In which of the substances the radioactive $^{14}CO_2$ is supposed to enter and how ? Give the various steps sequentially.
80. Compare C_4 and C_3 pathways of CO_2 fixation during photosynthesis. Give well labelled and schematic sketch of C_4 pathways.
81. What is photophosphorylation ? Distinguish between cyclic and non-cyclic photophosphorylations. Give schematic sketches of both the pathways.
82. Describe the light reaction of photosynthesis with special reference to non-cyclic and cyclic photophosphorylation.
83. (a) Give a schematic outline of photorespiration.
(b) Differentiate between photophosphorylation and photorespiration.
84. Where does Calvin cycle take place in chloroplast ? Explain the cycle.
85. (a) Schematically represent non-cyclic photophosphorylation in Angiosperms, giving all the components.
(b) Why is the process called non-cyclic ?

Multiple Choice Questions (With Answers)

- (1) C_4 plants differ from C_3 plants with respect to (a) Number of ATP molecules consumed (b) First product (c) Substrate which accepts carbon dioxide (d) All the above. (AFMC 1997)
- (2) The response of different organisms to environmental rhythms of light and darkness is called (a) Vernalization (b) Phototaxis (c) Photoperiodism (d) Phototropism (CBSE 1998)
- (3) Which one of the following statements about cytochrome P_{450} is wrong ? (a) It has an important role in

- metabolism. (b) It contains iron. (c) It is an enzyme involved in oxidation. (d) It is a coloured cell.
- (4) Pigments of PS I occur in (a) appressed part of granal thylakoids (b) stromal thylakoids and non-appressed parts of granal thylakoids. (c) both appressed and non-appressed parts of granal thylakoids (d) stroma. (CBSE 1998)
 - (5) In photosynthesis photolysis of water is used in (a) reduction of NADP^+ (b) oxidation of NADP^+ (c) oxidation of FAD (d) none of the above. (BHU 1998)
 - (6) The pigment present universally in all green oxygenic plants is (a) chlorophyll a (b) chlorophyll b (c) chlorophyll c (d) chlorophyll m. (CPMT 1998)
 - (7) Carbon dioxide is fixed in (a) light reaction (b) dark reaction (c) aerobic respiration (d) anaerobic respiration. (MP PMT 1999)
 - (8) For fixing one molecule of CO_2 in Calvin cycle, are required (a) $3\text{ATP} + 1\text{NADPH}_2$ (b) $3\text{ATP} + 2\text{NADPH}_2$ (c) $2\text{ATP} + 3\text{NADPH}_2$ (d) $3\text{ATP} + 3\text{NADPH}_2$. (MP PMT 1999)
 - (9) Enzyme required in early CO_2 fixation in C_4 fixation in C_4 cycle is (a) RuBP carboxylase (b) RuBP oxygenase (c) PEP carboxylase (d) PGA dehydrogenase. (CBSE 2000)
 - (10) Which of the following occurs in dark reaction of photosynthesis ? (a) Formation of ATP (b) Release of O_2 (c) Release of H_2 (d) Synthesis of PGAL. (CBSE 2000)
 - (11) Chemical which absorbs light energy and changes it to chemical energy is (a) xanthophyll (b) chlorophyll a (c) chlorophyll b (d) chlorophyll c. (AFMC 2000)
 - (12) Hill used a dye for his famous Hill reaction (a) sulphur green (b) eosine (c) methylene blue (d) dichlorophenol indophenol. (AIIMS 2000)
 - (13) Carotenes protect plants from (a) photo-oxidation (b) desiccation (c) photorespiration (d) photosynthesis. (Har. PMT 2000)
 - (14) Pigment system connected with oxidation of water is (a) PS I (b) PS II (c) Phycobilisome (d) Carotenoids. (Har. PMT 2000)
 - (15) ATP molecules required to synthesise one molecules of glucose by C_4 pathways are (a) 12 (b) 18 (c) 24 (d) 30 (e) 36. (JIPMER 2000)
 - (16) Scientist awarded nobel prize in 1960 for tracing the path of carbon is (a) Hatch (b) Huber (c) Calvin (d) Ruben (e) Blackmann. (Kerala 2000)
 - (17) Photolithotrophs (Photoautotrophs) obtain energy from (a) radiations and carbon from inorganic compounds (b) radiations and carbon from organic compounds (c) organic compounds (d) inorganic compounds. (Kerala 2001)
 - (18) Who was the first to show that solar energy is used in synthesis of organic matter (a) Bayer (b) Mayer (c) Hill (d) Van steil. (CBSE 2001)
 - (19) First transitory biochemical produced in reaction between carbon dioxide and ribulose biphosphate is (a) DiHAP (b) 2-carboxy 3-keto 1,5-biphosphoribitol (c) PGA (d) PGAL. (Har. PMT 2002)
 - (20) Red colour of Tomato is due to (a) Phytochrome (b) Anthocyanin (c) Chromatochrome (d) Lycopene. (JK 2002)
 - (21) Electrons passed out by PS II are replaced from (a) H_2O (b) O_2 (c) CO_2 (d) None of the above. (DPMT 2002)
 - (22) Passage of materials through vascular tissues is called (a) Transduction (b) Transcription (c) Transpiration (d) Translocation. (BHU 2002)
 - (23) Energy obtained from light reaction is transferred to dark reaction in the form of (a) ATP (b) ADP (c) RuBP (d) Chlorophyll. (CBSE 2002)
 - (24) Which one is correct (a) PSII has oxygen producing complex (b) NADPH is assimilatory power (c) $\text{PEP} \rightarrow \text{pyruvate}$ produces substrate level phosphorylation (d) all the above. (Manipal 2002)
 - (25) Process of loss of energy is (a) Fluorescence (b) photooxidation (c) photophosphorylation (d) photolysis. (JIPMER 2002)
 - (26) Photosynthesis is maximum in (a) Green light (b) Red light (c) Continuous light (d) very high light. (AFMC 2003)
 - (27) In sugarcane, CO_2 is fixed in malic acid with the help of enzyme (a) PEP carboxylase (b) RuBP carboxylase (c) Ribulose phosphate kinase (d) Fructose phosphatase. (CBSE 2003)
 - (28) Photorespiration in C_3 plants begins from (a) glycerate (b) phosphoglycolate (c) phosphoglycerate (d) glycine. (AIIMS 2003)
 - (29) Hill reaction requires (a) absence of water (b) total darkness (c) high attitude (d) presence of

- Ferricyanide.
- (30) Photolysis of water by isolated chloroplasts was demonstrated by (a) Hill (b) Von Niel (c) Liebig (d) Calvin. (AIIMS 2003)
- (31) Which is not an electron carrier (a) CoA (b) Cyt c (c) Cyt a (d) H_2O (e) Cyt a_3 . (AIEEE 2004)
- (32) As compared to sun plants, plants adapted to low light intensity possess (a) high rate of CO_2 fixation (b) larger photosynthetic unit (c) more extended root system (d) spiny leaves. (Kerala 2004)
- (33) Carbon in CO_2 is radioactively labelled. The product in which radioactive carbon can be traced in C_3 plants is (a) PEP (b) RuBP (c) PGAL (d) PGA. (CBSE 2004)
- (34) Which one has Kranz anatomy ? (a) Maize (b) Wheat (c) Rice (d) Potato. (Manipal 2005)
- (35) Which one is a CAM plant ? (a) Maize (b) Pineapple (c) Onion (d) Pea. (HPPMT 2005)
- (36) Wavelength of PAR is (a) 340–450 nm (b) 400–700 nm (c) 500–600 nm (d) 450–950 nm. (Orissa 2005)
- (37) Solarisation is (a) Formation of chlorophyll (b) Effect of solar light (c) Destruction of chlorophyll (d) Utilisation of sunlight. (DPMT 2005, 2008, CBSE 2005)
- (38) In photosystem I, the first electron acceptor is (a) An Fe-S protein (b) Ferredoxin (c) Cytochrome (d) Plastocyanin. (BHU 2005)
- (39) Chloroplasts do not have grana in (a) Bundle sheath cells of C_3 plants (b) Bundle sheath cells of C_4 plants. (c) Mesophyll cells of all plants. (d) Mesophyll cells of C_4 plants. (CBSE 2006)
- (40) Which one provides electrons for reduction reaction of photosynthesis (a) Chlorophyll (b) Cytochrome (c) NADPH (d) Water. (KCET 2006)
- (41) Electron acceptor of PS II is (a) FRS (b) Cyt b (c) PQ (d) $NADP^+$. (CET Chd 2006)
- (42) In C_4 plants malic acid is formed in the cells of (a) mesophyll (b) epidermis (c) phloem (d) bundle sheath. (CBSE 2007)
- (43) Succulents carry out photosynthesis (a) CAM pathway (b) C_4 pathway (c) C_3 pathway (d) C_2 pathway. (CBSE 2007, 2008)
- (44) Which wavelength is inactive in PS I (a) 780 nm (b) 680 nm (c) 680 nm (d) 690 nm (d) 550 nm. (JKCME 2007)
- (45) Law of limiting factors was proposed by (a) Hill (b) Arnon (c) Blackmann (d) Von Mayer. (DPMT 2008)
- (46) In C_3 plants, first stable product of photosynthesis during dark reaction is (a) Malic acid (b) 3-phosphoglyceric acid (c) Oxaloacetic acid (d) Phosphoglyceraldehydes. (AFMC 2008)
- (47) Electrons from excited chlorophyll molecules of photosystem II are accepted first by (a) Ferredoxin (b) Quinone (c) Cytochrome b (d) Cytochrome f. (CBSE 2008)
- (48) About 70% of global carbon occurs in (a) Forests (b) Grassland (c) Agroecosystems (d) Oceans. (CBSE 2008)
- (49) Chloroplast stroma contains (a) chlorophyll (b) Light dependent reaction enzymes (c) Light independent reaction enzymes (d) Ribosomes. (CBSE 2009)
- (50) Cyclic photophosphorylation forms (a) NADPH (b) ATP (c) ATP and NADPH (d) ATP, NADPH and O_2 . (CBSE 2009)
- (51) Chloroplast dimorphism is a characteristic feature of (a) plants with Calvin cycle (b) C_4 plants (c) all plants (d) only in algae. (W.B. 2010)
- (52) PGA as the first CO_2 fixation product was discovered in photosynthesis of (a) Gymnosperm (b) Angiosperm (c) Alga (d) Bryophyte. (CBSE 2010)
- (53) A reduction of O_2 evolution occurs when light wavelength is (a) more than 680 nm (b) less than 680 nm (c) less than 660 nm (d) about 560 nm. (JKCME 2011)
- (54) Of the total solar radiation the proportion of PAR is (a) less than 50% (b) more than 80% (c) about 70% (d) about 60%. (DPMT 2011)
- (55) CAM helps the plants in (a) reproduction (b) secondary growth (c) conserving water (d) disease resistance. (CBSE 2011)

Assertion and Reason Type Questions

In each of the following questions two statements are given, one is Assertion (A) and other is Reason (R). For the (A) and (R) statements, mark the correct answer as—

- (A) If both A and R are true and R is the correct explanation of A
 (B) If both A and R are true and R is not the correct explanation of A

(C) If A is true but R is false.

(D) If both A and R are false.

- (1) **Assertion.** In fluorescence, chlorophyll emits short wave radiations.
Reason. Far more energy is absorbed by chlorophyll than required for photosynthesis.

(A) (B) (C) (D)

- (2) **Assertion.** The acceptor molecule of Calvin cycle is ribulose 1,5-biphosphate.
Reason. It was discovered by Basham.

(A) (B) (C) (D)

- (3) **Assertion.** Dark fixation of CO_2 in CAM plants is called ocification.
Reason. It produces malic acid.

(A) (B) (C) (D)

- (4) **Assertion.** Submerged plants get CO_2 in the form of carbonates and bicarbonates.
Reason. Stomata are not present in submerged plants.

(A) (B) (C) (D)

- (5) **Assertion.** A plant girdled upto bast shows signs of degeneration of lower portion.
Reason. Water transportation will cease.

(A) (B) (C) (D)

- (6) **Assertion.** In bacteria photosynthesis occurs by utilising wavelengths longer than 700 nm.
Reason. Reaction centre is B_{890} .

(A) (B) (C) (D)

- (7) **Assertion.** Six molecules of CO_2 , 12 molecules of $\text{NADPH} + \text{H}^+$ and 18 ATP are required to form one hexose molecule.
Reason. Light reaction produces ATP and $\text{NADPH} + \text{H}^+$

(A) (B) (C) (D)

- (8) **Assertion.** Cyclic pathway of photophosphorylation first appeared in some eubacterial species.
Reason. Oxygen started accumulating in the atmosphere after noncyclic pathway of photosynthesis evolved.

(A) (B) (C) (D)

- (9) **Assertion.** C_4 photosynthetic pathway is more efficient than C_3 pathway.
Reason. Photorespiration is suppressed in C_4 plants.

(A) (B) (C) (D)

- (10) **Assertion.** The atmospheric concentration of CO_2 at which photosynthesis just compensates for respiration is referred to as CO_2 -compensation point.

Reason. CO_2 -compensation point is reached when the amount of CO_2 uptake is less than generated through respiration because the level of CO_2 is more than that required for achieving CO_2 -compensation point.

(A) (B) (C) (D)

- (11) **Assertion.** Under conditions of high intensity and limited CO_2 supply, photorespiration has a useful role in protecting the plants from photooxidative damage.

Reason. If enough CO_2 is not available to utilize light energy for carboxylation to proceed, the excess energy may not cause damage to plants.

(A) (B) (C) (D)

- (12) **Assertion.** Photosynthetically C_4 plants are less efficient than C_3 plants.

Reason. The operation of C_4 pathway requires the involvement of only bundle sheath cells.

(A) (B) (C) (D)

ANSWERS

Multiple Choice Questions

- (1) —d (2) —c (3) —d (4) —b (5) —a (6) —a (7) —b (8) —b (9) —c (10) —d
 (11) —b (12) —d (13) —a (14) —b (15) —d (16) —c (17) —a (18) —b (19) —b (20) —d
 (21) —a (22) —d (23) —a (24) —d (25) —a (26) —c (27) —a (28) —b (29) —d (30) —a
 (31) —d (32) —b (33) —d (34) —a (35) —b (36) —b (37) —c (38) —a (39) —b (40) —d
 (41) —c (42) —a (43) —a (44) —a (45) —c (46) —b (47) —b (48) —d (49) —c (50) —b
 (51) —b (52) —c (53) —a (54) —a (55) —c

Assertion and Reason Type Questions

- (1) —D (2) —B (3) —A (4) —D (5) —C (6) —B (7) —B (8) —B (9) —A (10) —C
 (11) —A (12) —D